The effect of β-glucan prebiotic fibre (oats) on the gut microbiome of chronic kidney disease patients (Stage IV and V) and impact on kidney function

by

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Research team and contributions to the PhD project

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Summary

Background: Chronic kidney disease (CKD) is increasing in global prevalence and has many nutritional complications. Increasing evidence suggests that gut dysbiosis is involved in CKD progression through various mechanisms including intestinally derived uraemic toxins, dietary, and immune-mediated factors. Therefore, modulating the gut microbiome may improve outcomes in CKD. The aim of this research project was to investigate the effect of a ß-glucan prebiotic supplement on kidney outcomes, uraemic toxins and the gut microbiome in predialysis CKD participants.

Methods: This study was a randomised controlled intervention study over 18 weeks, performed at Tygerberg Hospital predialysis clinic in Cape Town, South Africa. There was a pre-randomisation period of four weeks where participants were counselled on a CKD diet before being randomised. At randomisation, the intervention group received the ß-glucan supplement and continued the diet, while the control group continued with the diet only. There were follow-ups at weeks 4, 8 and 14 after randomisation. The objectives were to assess nutritional status, kidney function, plasma levels of uraemic toxins and gut microbiota using 16S rRNA sequencing at pre-randomisation. Additionally, differences in these outcomes were measured at randomisation baseline (week 0), week 8 and week 14 between the intervention and the control groups. Anthropometrical measurements were done which included weight, height, waist circumference, mid-upper arm circumference and triceps. Clinical investigations included investigating for oedema as well as gastrointestinal symptom measurement. Stool consistency was described using the Bristol Stool Score (BSS). Dietary intake was measured using a quantified food frequency questionnaire (QFFQ) and a dietary adherence score sheet. Although most of the investigations was done locally, the uraemic toxins analysis was performed at the nephrology laboratories at the University of Ghent in Belgium, while the gut microbiome analysis was performed at VIB laboratories (Leuven, Belgium). Statistical analysis was performed using IBM®SPSS®version 26/27 and R Statistical Software.

Results: Seventy participants were enrolled in the study at the pre-randomisation visit. The mean age of the participants was 41.7 ± 11.8 years, with a slight predominance of females (53%). Most participants were unemployed, earning less than US$126 per month. Hypertension was the main cause of kidney failure and most participants were in stage 5 CKD. A very high prevalence of overweight (30%) and obesity (36%) was found at pre-

randomisation, with a low prevalence of undernutrition (3%). Abdominal obesity was found in 60% of participants. Dietary assessment showed an unhealthy dietary pattern. After four weeks, 59 participants were randomised. The diet intervention resulted in significant nutritional changes in participants after four weeks, while uraemic toxins remained unchanged. There was a significant reduction in body mass index ($P < 0.006$) and waist circumference ($P < 0.001$). Almost all dietary intake variables were significantly reduced and there was a high dietary adherence. Serum total cholesterol ($P < 0.045$) and triglyceride levels ($P < 0.017$) were also reduced. After randomisation to either the ß-glucan prebiotic or the diet, kidney function did not significantly change. However, there was a significant reduction in uraemic toxins in free IxS at week 8 ($P = 0.003$) and week 14 ($P < 0.001$), total and free *p*CG ($P < 0.001$, $P < 0.001$, respectively) and free *p*CS ($P = 0.006$) at week 14. There were no significant changes in dietary intake, clinical symptoms or anthropometry during the trial. The gut microbiome revealed that two enterotypes were prevalent, namely the *Bacteroides*2 and *Prevotella* enterotypes. The inter-individual Bray–Curtis distance (ß-diversity) was significantly higher in the control group than the intervention group at baseline ($P < 0.0001$), week 8 ($P < 0.0001$) and week 14 ($P = 0.02$). There were no differences in relative abundance of genera between groups. The redundancy analysis showed a few factors significantly affected the gut microbiome: these included triglyceride levels ($P < 0.001$), cause of kidney failure ($P < 0.001$), gender ($P < 0.001$), body mass index ($P = 0.002$), high- density lipoprotein ($P < 0.001$) and the prebiotic intervention ($P = 0.002$).

Conclusion: While four weeks of the diet resulted in some nutritional changes in participants before randomisation, it did not affect other outcomes of the study. Once randomised, the prebiotic did not significantly affect kidney function, while it significantly reduced uraemic toxins and the gut microbiome, according to the RDA analysis. The ß-glucan prebiotic therefore had some beneficial effects on outcomes in CKD participants.

Opsomming

Agtergrond: Chroniese niersiekte (CNS) se voorkoms wêreldwyd neem toe en het talle voedingskomplikasies. Toenemende getuienis dui daarop dat dermdisbiose deur verskillende meganismes, insluitend intestinaal afgeleide uremiese toksiene, en dieet- en immuun-bemiddelde faktore, by die vordering van CNS betrokke is. Die modulering van die dermmikrobioom kan dus uitkomste in CNS verbeter. Die doel van hierdie navorsingsprojek was om die effek van ’n ß-glukaan- prebiotiese supplement op nieruitkomste, uremiese toksiene en die dermmikrobioom by voordialise-CNS-deelnemers te ondersoek.

Metodes: Hierdie studie was ’n ewekansige gekontroleerde intervensiestudie oor 18 weke wat by die Tygerberg Hospitaal se voordialisekliniek in Kaapstad, Suid-Afrika uitgevoer is. Daar was ’n voor-ewekansigingsperiode van vier weke waar deelnemers dieetonderriging ontvang het oor ’n CNS-dieet voordat hulle ewekansig is. Met ewekansiging het die intervensiegroep die ß-glukaansupplement ontvang en met die dieet voortgegaan, terwyl die kontrolegroep slegs met die dieet voortgegaan het. Daar was opvolgbesoeke in week 4, 8 en 14 ná ewekansiging. Die doelwitte was om voedingstatus, nierfunksie, plasmavlakke van uremiese toksiene en dermmikrobiotika met behulp van 16S rRNS-volgordebepaling met voor-ewekansiging te evalueer. Verder is verskille in hierdie uitkomste met die ewekansigingsbasislyn (week 0), week 8 en week 14 tussen die intervensie- en kontrolegroep gemeet. Antropometriese metings is gedoen, wat gewig, lengte, middelyfomtrek, mid-bo-arm omtrek en triseps ingesluit het. Kliniese ondersoeke het ’n ondersoek vir edeem asook die meting van gastroïntestinale simptome ingesluit. Stoelgangtekstuur is met behulp van die Bristol-stoelgangtelling (Bristol Stool Score) beskryf. Dieetinname is met behulp van ’n voedselfrekwensievraelys gedoen en dieetnakoming is gemeet. Hoewel die meeste van die ondersoeke plaaslik gedoen is, is die uremiesetoksienontleding by die nefrologielaboratoriums van die Universiteit van Ghent in België gedoen, terwyl die dermmikrobioomontleding by VIB-laboratoriums (Leuven, België) gedoen is.

Resultate: Sewentig deelnemers is met die voor-ewekansigingsbesoek vir die studie ingeskryf. Die gemiddelde ouderdom van die deelnemers was 41.7 ± 11.8 jaar, met ’n geringe oorheersing van vroue (53%). Die meeste deelnemers was werkloos en het minder as US$126 per maand verdien. Hipertensie was die vernaamste oorsaak van nierversaking en die meeste deelnemers was op stadium 5 CNS.’n Baie hoë voorkoms van oorgewig

(30%) en vetsug (36%) is met voor-ewekansiging gekry, met ’n lae voorkoms van ondervoeding (3%). Abdominale vetsug is by 60% van die deelnemers gevind. ’n Dieetevaluering het ’n ongesonde dieetpatroon getoon. Ná vier weke is die deelnemers ewekansig. Die dieetintervensie het ná vier weke tot belangrike voedingsveranderinge by deelnemers gelei, met uremiese toksiene wat onveranderd gebly het. Daar was ’n beduidende vermindering in die liggaamsmassa-indeks ($P < 0.006$) en middelomtrek ($P < 0.001$). Feitlik alle dieetinnameveranderlikes is beduidend verlaag en daar was ’n hoë nakoming van die dieet. Serum-totale cholesterol ($P < 0.045$) en trigliseriedvlakke ($P < 0.017$) is ook verlaag. Nadat deelnemers ewekansig is na óf die ß-glukaanvoorbiotikum óf die dieet, het die nierfunksie nie beduidend verander nie. Daar was egter ’n beduidende verlaging in uremiese toksiene in vrye IxS in week 8 ($P = 0.003$) en week 14 ($P < 0.001$), totale en vrye *p*CG ($P < 0.001$ en $P < 0.001$, onderskeidelik) en vrye *p*CS ($P = 0.006$) in week 14. Daar was geen beduidende veranderings in dieetinname, kliniese simptome of antropometrie tydens die studie nie. Die dermmikrobioom het getoon dat twee enterotipes voorgekom het, naamlik die *Bacteroides* 2- en *Prevotella*-enterotipe. Die interindividuele Bray-Curtis-afstand (ß-diversiteit) was beduidend hoër by die kontrolegroep as by die intervensiegroep, met basislyn ($P < 0.0001$), week 8 ($P < 0.0001$) en week 14 ($P = 0.02$). Daar was geen verskille in relatiewe oorvloed van genera tussen groepe nie. Die oortolligheidsontleding het getoon dat ’n paar faktore die dermmikrobioom beduidend beïnvloed het: dit het trigliseriedvlakke ($P < 0.001$), die oorsaak van nierversaking ($P < 0.001$), geslag ($P < 0.001$), liggaamsmassa-indeks ($P = 0.002$), hoëdigtheid-lipoproteïen ($P < 0.001$) en die prebiotika intervensie ($P = 0.002$) ingesluit.

Gevolgtrekking: Hoewel vier weke van die dieet voor ewekansiging tot ’n paar voedingsveranderings by deelnemers gelei het, het dit nie ander uitkomste van die studie geraak nie. Nadat die deelnemers ewekansig is, het dit nie nierfunksie beduidend geraak nie, terwyl dit uremiese toksiene en die dermmikrobioom volgens die RDA-ontleding beduidend verminder het. Die ß-glukaanprebiotikum het dus ’n paar voordelige uitwerkings op uitkomste by CNS-deelnemers gehad.

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Glossary

Chronic kidney disease	Structural or functional abnormalities of the kidney. Functionally, glomerular filtrationrate of < 60ml/min/1.73 m^2 or albuminuria of greater than 30 mg per day for more than 3 months [1].
Enterotypes	“Densely populated areas in a multidimensional space of community composition” should not be seen as clusters but a way of stratifying samples [2].
Gut dysbiosis	Alterations in the function and composition of the gut microbiota due to host microbial imbalance [3].
Gut microbiome	Collective genetic material of the microbial community in the gut [4].
Kidney function	Refers to the excretory, metabolic and hormonal functions of the kidney [5]. For the objectives of the study, the excretory functions of the kidney are referred to.
Nutritional assessment	Nutritional assessment includes the collecting of anthropometry, biochemistry, clinical signs, dietary intake and food security [6].
Predialysis	Stage 5 chronic kidney disease not yet requiring dialysis.
Pipelines	A computer program that combines several programs in a certain way to analyse complex data [7].

Prebiotic	Prebiotics are non-viable food components that confers a health benefit on the host by modulating the gut microbiota [8].
Principal coordinate analysis (PCoA)	A method to explore data structure in a graph; if it is unconstrained by environmental factors it is referred to as principal coordinates analysis [7].
Probiotic	Probiotics are live, selected strains of bacteria that when administered in adequate amounts confer health benefits [8]
Simplified dietary advice in chronic kidneydisease (CKD)	Dietary advice that has been simplified for CKD based on the literature, advising healthy eating and limiting additives, processed food and salt [9].
Uraemic toxins	Retention of solutes that are normally cleared by the kidney that negatively affect biologic functions; these include indoxyl sulfate, *p*-cresyl sulfate, *p*- glucoronide sulfate, indoxyl acetic acid and trimethylamine-N-oxide [10].
Redundancy analysis (RDA)	Constrained ordination that combines principal ordinate analysis and regression; it shows whether microbiota data are constrained by clinical factors [11].
Sequencing (16S ribosome rDNA)	Amplicon sequencing methods focusing on specific gene markers (16S ribosome DNA) to study bacterial composition [7].
Synbiotic	It is the combination of synergistically acting prebiotics and probiotics [8]

Abbreviations

Abbreviation	Description
ACR	Albumin to creatine ratio
ASVs	Amplicon sequence variants
AXOS	Arabinoxylan oligosaccharides
BSH	Bile salt hydrolase
BMI	Body mass index
BSS	Bristol Stool Scale
CRP	C-reactive protein
CVD	Cardiovascular disease
CKD	Chronic kidney disease
DASH	Dietary Approaches to Stop Hypertension
DMM	Dirichlet multinomial mixtures
ESKD	End-stage kidney disease
EPO	Erythropoietin
GIT	Gastrointestinal
GFR	Glomerular filtration rate
H_2S	Hydrogen sulphide

HSDA	sodium *N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline
HTs	High-throughput sequencing
IAA	Indoxyl acetic acid
IxS	Indoxyl sulfate
ISE	Ion sensitive electrodes
ISRNM	International Society of Renal Nutrition and Metabolism
KDIGO	Kidney Disease Improving Global Outcomes
KDOQI	Kidney Disease Outcomes Quality Initiative
LMIC	Low- and middle-income country
MW	Molecular weight
NADH	Nicotinamide adenine dinucleotide
NHRD	National Health Research Database
OTUs	Operational taxonomic units
*p*CS	*p*-Cresyl sulfate
*p*CG	*p*-Cresyl glucuronide
pCoA	Principal coordinate analysis
PEG	polyethylene glycol
PI	Principal investigator
PTH	Parathyroid hormone
QFFQ	Quantified food frequency questionnaire

RA	Research Assistant
RCT	Randomised controlled trial
RDA	Redundancy analysis
ROS	Reactive oxygen species
SCFAs	Short-chain fatty acids
SGA	Subjective global assessment
TMNO	Trimethylamine-N-oxide
VLDL	Very-low density lipoprotein

CHAPTER 1: INTRODUCTION

1.1 Rationale for the study

Chronic kidney disease (CKD) is a non-communicable disease with an increasing global prevalence of between 9 and 13% [12, 13]. In sub-Saharan Africa it has a slightly higher prevalence of 14% [14]. This is contributed to by the increased prevalence of hypertension and diabetes, which are two of the main causes of CKD [15]. Obesity is a risk factor for these chronic conditions and has been associated with CKD development [15, 16]. CKD has many metabolic and nutritional complications requiring intensive medical interventions which are expensive and compromise patients' quality of life [17]. Cardiovascular disease is the leading cause of mortality in CKD, despite controlling for traditional risk factors [18]. There are many challenges in South Africa in providing optimal CKD care [19]. These include limited access to renal replacement treatment in the public sector, and a lack of resources, including financial resources and skilled personnel such as nephrologists, nurses, technicians and dietitians [19, 20]. In addition, there are inequities in the provision of healthcare between the public and private sector, with the private sector having grown substantially despite serving a minority of the population [19]. Therefore, it is imperative that interventions are aimed at reducing the prevalence, as well as delaying the progression of CKD. If dietary interventions are instituted at early stages of the disease, it can be a key strategy to delay the need for dialysis in an already resource-limited setting and improve the quality of life in this population. The modulation of the gut microbiome in CKD has in recent years been shown to be a potential therapeutic option to reduce the progression of the disease.

Figure 1.1 shows the various causes of gut dysbiosis which are discussed below.

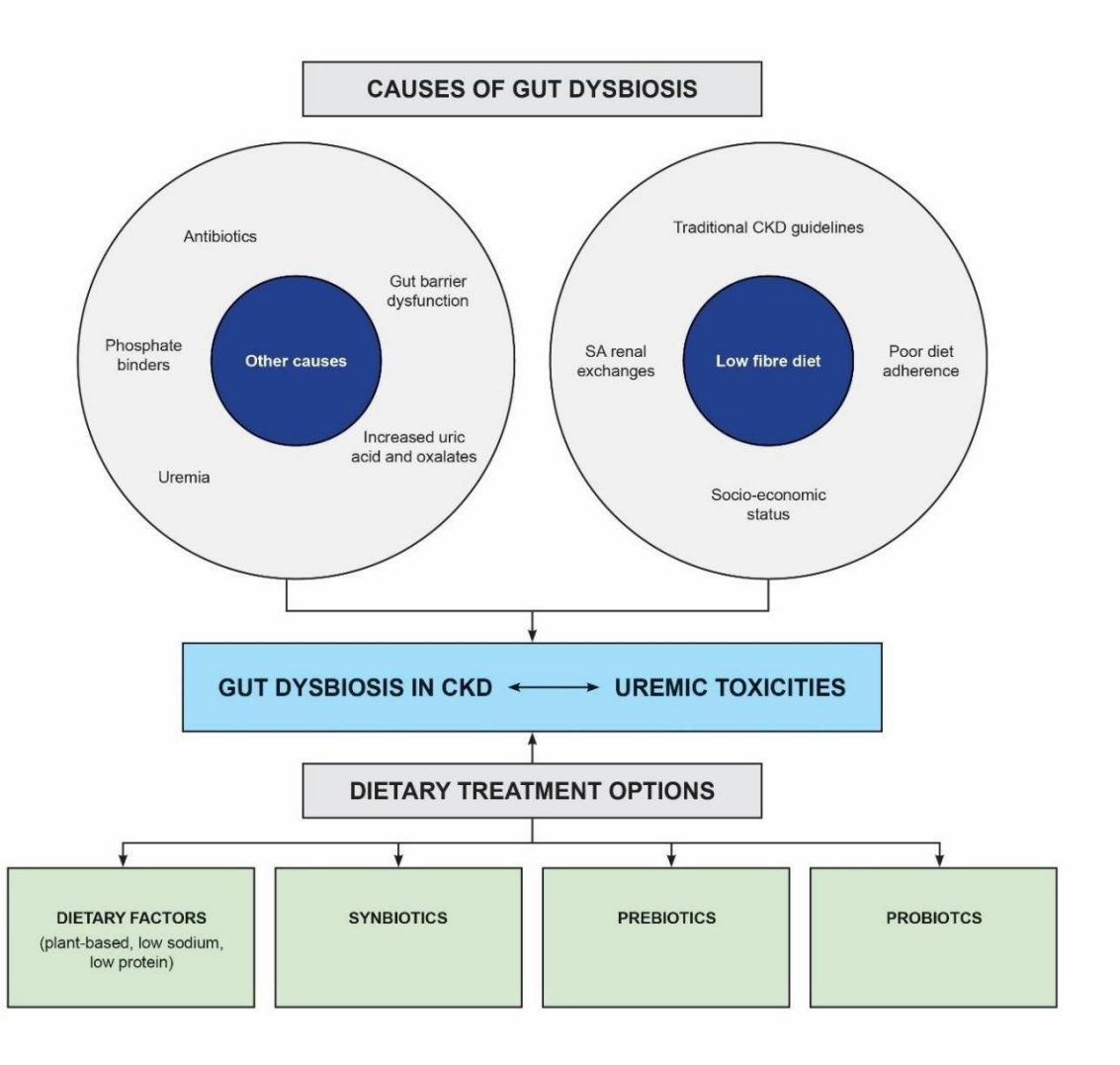

Figure 1.1: Causes of and treatment options for gut dysbiosis

Despite evidence showing dietary modifications slow disease progression, adherence to dietary guidelines have been low owing to the restrictive nature of the renal diet [21, 22]. The traditional diet for CKD prescribes low intakes of fruits, vegetables and wholegrains; this diet minimises potassium and phosphorous consumption, thereby limiting fibre intake [23]. In South Africa, CKD patients are traditionally managed using the South African renal exchange lists. This is an extensive list of 11 food groups, with varying amounts of macronutrients and micronutrients including potassium, sodium, and phosphate. Diet sheets were also developed from some of these exchanges to allow patients to select the appropriate foods. These guidelines and exchange lists are challenging to follow, which may result in poor adherence. In addition, socio-economic status may also limit the purchase of fibre-rich foods.

The resultant low-fibre intake and other factors affect the gut microbiome and contribute to gut dysbiosis and CKD progression though several mechanisms, including gut-derived uraemic toxins, immune-mediated products, and neuroendocrine mediated substances [24, 25]. The other causes of dysbiosis resulting from the biochemical imbalance caused by CKD include gut barrier dysfunction, increased levels of uric acid and oxalates, use of phosphate binders and antibiotics [24].

Uraemic toxins such as indoles and phenols have shown to cause many complications such as inflammation, cardio-renal syndrome, bone mineral disease and impaired cognitive function, in essence all the organ systems that are affected in CKD [4, 26]. These uraemic toxins have also been shown to increase cardiac mortality [26]. Studies have shown distinct differences in the gut microbiome in CKD, with short-chain fatty acid organisms being lower and proteolytic organisms being higher in CKD compared with healthy individuals [27]. Very recent experimental studies show that plant-based diets are associated with a reduction in uraemic toxins and changes in the gut microbiome [28]. Studies using prebiotics, probiotics and synbiotics in improving gut dysbiosis show varying results [29, 30]. Although these supplements seem promising, studies show limited efficacy owing to the lack of randomised control trials, small sample sizes and short study periods [29]. Prebiotics offer the most natural and affordable option. ß-glucan prebiotic fibre found in oats has many beneficial physiological properties that improve risk factors associated with CKD such as lowering cholesterol levels [31] and uraemic toxins [32]. The beneficial properties include its lipid solubility, the presence of resistant starches and the many phenolic compounds [33]. These properties make it a valuable therapeutic option to explore in CKD. Dietary adaptations and prebiotics may therefore be a more affordable option in South Africa to modulate the gut microbiome, uremic toxins and kidney function.

1.2 Significance of the study

Gut dysbiosis in CKD occurs for various reasons and it has been shown that the composition of the gut microbiome in CKD is very different from that of healthy controls [27]. There are limited randomised clinical trials investigating the effect of prebiotics on kidney function, uraemic toxins and the gut microbiome. There is only one trial to date which showed favourable outcomes on the gut microbiome and uraemic toxins, although the supplement used was a synbiotic, which is a combination of prebiotics and probiotics [34]. Some trials on prebiotics in predialysis patients have shown some significant reductions in uraemic toxins and kidney function; however these studies have not

investigated the effect of prebiotics only on the gut microbiome [29]. There are no trials in South Africa that have investigated the gut microbiome or uraemic toxins in CKD patients. This single-blinded, randomised controlled trial may provide evidence of the effect of ß-glucan prebiotic on kidney function, uraemic toxins and the gut microbiome in CKD predialysis patients. These outcomes are all interrelated and may provide insight into the mechanisms behind gut dysbiosis in CKD.

1.3 Research question

How does the inclusion of ß-glucan prebiotic fibre (oats) in addition to a CKD diet affect the gut microbiome, uraemic toxins and kidney function of CKD predialysis patients over 14 weeks compared with the CKD diet alone?

1.4 Aims and objectives

This study aimed to investigate the effect of a ß-glucan prebiotic fibre supplement (oats) on the gut microbiome, uraemic toxins, and kidney function in CKD stage 3 to 5 patients attending the Tygerberg Hospital outpatient CKD clinic by means of a randomised controlled trial (RCT).

Primary objectives:

1.4.1 To describe the pre-randomisation characteristics of the gut microbiome profile and uraemic toxins in CKD patients on admission to the study. This objective is addressed in Chapter 6 and 7.

1.4.2 To describe the pre-randomisation nutritional status and biochemical profile on admission to the study. This objective will be addressed in Chapter 5.

1.4.3 To educate the patients on a CKD diet at the pre-randomisation phase and evaluate dietary adherence based on dietary education given during the study. This is addressed in Chapters 5, 6 and 7.

1.4.4 To compare the effect of ß-glucan prebiotic fibre on the outcomes below in the intervention (CKD diet and ß-glucan prebiotic fibre) and control group (CKD diet only) at the following time points, 8 and 14 weeks from the time they were randomised. This objective is addressed in Chapter 7:

- Gut microbiome profile
- Kidney function
- Uraemic toxin levels

- Other biochemical parameters including lipid function tests and an inflammatory marker

1.5 Hypotheses

The following null hypotheses were tested:

Hypothesis 1

There is no difference in the gut microbiome in CKD patients consuming prebiotic ß-glucan fibre (oats) compared with a CKD diet.

Hypothesis 2

There is no difference in kidney function progression in CKD patients consuming prebiotic ß-glucan fibre (oats) compared with a CKD diet.

Hypothesis 3

There is no difference in the uraemic toxin levels in CKD patients consuming prebiotic ß-glucan fibre (oats) compared with a CKD diet.

1.6 Overview of chapters

This thesis has the format of a hybrid thesis and consists of the following chapters. Chapter 1 comprises the introduction, research question, aims and objectives of the study.

Chapter 2 provides a literature review of key components of the study from a local and international perspective, to provide the background to the study.

Chapter 3 provides an overview of the methodology, since each article also has a methodology section.

Chapter 4 presents the first published article in the *Journal of Renal Nutrition* in July 2020 on the dietary infographic used to educate participants in the study. The title of the article is: “Keeping the Diet Simple and Natural in Chronic Kidney Disease: A South African-Based Dietary Infographic”.

Chapter 5 presents the second published article in *Nutrients* in November 2020 that describes the baseline data of the nutritional status of the participants. The title of the

article is “Obesity and Other Nutrition-Related Abnormalities in Predialysis Chronic Kidney Disease (CKD) Participants”.

Chapter 6 presents the third published article in the South African Journal of Clinical Nutrition (SAJCN) in February 2022. It presents the nutritional and uraemic toxin changes during the run-in period. The title of this article is “Effect of simplified dietary advice on nutritional status and uremic toxins in chronic kidney disease participants”.

Chapter 7 presents the main article of the study published in *Nutrients* in February 2022. It explains the effect of the ß-glucan prebiotic on kidney function, uraemic toxins and the gut microbiome. The title of the article is “The Effect of ß-Glucan Prebiotic on Kidney Function, Uraemic Toxins and the Gut Microbiome in Stage 3 to 5 Chronic Kidney Disease (CKD) Predialysis Participants: A randomized controlled trial”.

Chapter 8 comprises the discussion, where all the findings of the articles and how they relate to one another are critically discussed. Furthermore, their impact on practice and how they contribute to the current body of knowledge are elaborated.

Chapter 9 presents the conclusion, recommendations and study limitations.

Owing to the hybrid structure of the thesis, references are displayed per chapter, with definitions’ references included in the Introduction references.

1.7 Skills development

During the course of the PhD journey, I acquired the following research-related skills:

Development of dietary education material	Owing to the lack of appropriate dietary education materials that encouraged less restrictive diets, I developed a dietary infographic. This was developed after reviewing the literature and in conjunction with specialised CKD dietitian colleagues in March 2018.
Qiime 2 Course	This workshop involved working with this Pipeline to process data from high throughput 16S sequencing.

Uraemic toxins analysis observation	I had the opportunity to visit Ghent University laboratories to understand and observe the methods involved in the uraemic toxin analysis in October 2019. A few days were spent at the laboratory to observe step-by-step uraemic toxin analysis.
MetaCoNekt interface	Owing to the Covid-19 pandemic, a special program was developed by the researchers at VIB laboratories (Leuven, Belgium) to allow for the visualisation of the results of the gut microbiome. I had to prepare all the study data in the format needed for the MetaCoNekT interface, which was sent in December 2020. I learned the skill of using the program and visualising the gut microbiome data with regard to pCOA plots, relative abundances and comparisons of groups and various outcome variables to gut microbiome genera. Most of the gut microbiome interpretation and visualisation were done in 2021.
Funding acquisition	I applied for numerous funds during 2018–2020, including the Early Researchers Fund, the Harry Crossley Fund, the National Research Foundation Thuthuka Fund, the MJ Thompson Sabbatical Fund and the Oppenheimer Sabbatical Fund.
Statistical analysis	I analysed the statistics using IBM®SPSS® version 26/27 for the study with the guidance of statisticians, except for the gut microbiome statistical analysis.

Workshops	I attended the following workshops: Academic article writing in July 2020, Word for thesis formatting in August 2021.

1.8 Research contributions by the PhD student

Parts of the research study results have been presented at peer-reviewed conferences or published in peer-reviewed journals.

Peer-reviewed conference presentations

Ebrahim Z, Esau N, Cilliers L. South African chronic kidney disease (CKD) diet: Back to basics, keep it natural. European Federation of the Association of Dietetics (EFAD) Congress, Rotterdam, Netherlands, 28–29 September 2018.

Ebrahim Z. Prebiotics and the gut microbiome in chronic renal disease. Association of Dietetics in South Africa Seminar, Cape Town, 9 November 2019.

Ebrahim Z, Moosa MR, Blaauw R. An assessment of the nutritional status of predialysis participants. Stellenbosch University Annual Academic Day, 26–27 August 2020.

Ebrahim Z, Esau N. Keeping the diet simple and natural in chronic kidney disease: A South African-based dietary infographic. International Renal Nutrition and Metabolism (ISRNM) Webinar, 17 May 2021.

Ebrahim Z, Glorieux G, Moosa MR, Blaauw R. Effect of dietary intervention on nutritional status and intestinally generated uraemic toxins in chronic kidney disease. Stellenbosch University Annual Academic Day, 18 August 2021.

Ebrahim Z, Blaauw R, Moosa MR. Prevalence of overweight and obesity in chronic kidney disease stage 3–5 attending a predialysis clinic in Cape Town, South Africa. International Congress of Dietetics 2021, 1–3 September 2021.

Ebrahim Z, Glorieux G, Moosa MR, Blaauw R. Effect of dietary intervention on nutritional status and intestinally generated uraemic toxins in chronic kidney disease. ESPEN Virtual Conference, 9–12 September 2021.

Peer-reviewed publications (PhD related)

Ebrahim Z, Esau N, Cilliers L. Keeping the diet simple and natural in chronic kidney disease: A South African-based dietary infographic. *J Ren Nutr*. 2020;30(4):e58–65. https://doi.org/10.1053/j.jrn.2019.11.007

Ebrahim Z, Moosa MR, Blaauw R. Obesity and other nutrition related abnormalities in pre-dialysis chronic kidney disease (CKD) participants. *Nutrients*. 2020, 12(12):e3608 http://dx.doi.org/10.3390/nu12123608

Ebrahim Z, Glorieux G, Moosa M.R, Blaauw R. Effect of simplified dietary advice on nutritional status and uremic toxins in chronic kidney disease participants. *SAJCN.* 2022. 1-9. https://doi.org/10.1080/16070658.2021.2018788

Ebrahim Z, Proost S, Tito RY, Raes J, Glorieux G, Moosa M.R, Blaauw R. The effect of ß- glucan prebiotic on kidney function, uremic toxins and gut microbiome in stage 3 to 5 Chronic Kidney Disease (CKD) predialysis participants: A randomized controlled trial. *Nutrients*. 2022. 14, 805. https://doi.org/10.3390/nu14040805

Other conferences attended:

Federation of International Dietitians Symposium Dublin October 2019.
Stellenbosch University African Microbiome symposium November 2019.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The literature review commences by providing the background to chronic kidney disease (CKD), its prevalence, diagnosis, complications and treatment, thereby setting the scene for an understanding of the disease and how it may affect the nutritional status of CKD patients. The nutritional status of CKD patients is elaborated upon in terms of the assessment and prevalence of malnutrition; thereafter nutritional management, including nutrient requirements, dietary patterns and dietary adherence is outlined. A discussion of the gut microbiome follows by describing the microbiota species typically found in the gut in CKD and how dietary factors affect the microbiota. An analysis of the gut microbiome as well as how gut dysbiosis affects intestinally derived uraemic toxins and gut microbiota is presented. Finally, supplementation in terms of probiotics, prebiotics and synbiotics and how they affect kidney function, the gut microbiome and intestinally produced uraemic toxins in CKD is discussed.

2.2 CKD

CKD is the persistent presence of any functional and/or structural abnormalities of the kidney; functionality is associated with reduced glomerular filtration rate (GFR) of less than 60 ml/min/1.73 m^2 and/or albuminuria of greater than 30 mg per day for more than 3 months [1].

2.2.1 CKD prevalence and challenges

The global prevalence of CKD is between 9 and 13% [2, 3] with sub-Saharan Africa having an even higher prevalence, estimated to be 14% [4]. It is associated with an increased economic burden on health systems and it is an independent risk factor for cardiovascular disease (CVD) [3].

The major complications of advanced CKD include cardiovascular disease, anaemia, bone-mineral disorders, fractures, and cognitive decline [5].

The South African Renal Registry [6] reports that the most prevalent known causes of CKD in South Africa are unknown (31.9%), hypertensive diseases (31.5%), diabetic nephropathy (15.3%) and glomerulonephritis (10.3%). This differs somewhat from more developed countries where the main causes are diabetes and hypertension [5]. The difference in developed countries relates to less infectious diseases, increased prevalence of chronic diseases, decreased birth rates and increased life expectancy. In low- and middle-income countries (LMICs), infectious diseases still predominate.

There is limited access to safe water, while pesticides, herbal environmental pollutants, analgesic abuse and increased food additives contribute to the burden of kidney disease [5].

South Africa faces many challenges in providing optimal care for CKD patients. These include limited resources, and lack of skilled personnel across various disciplines, including dietitians, nephrologists, nurses and technicians. Other problems include inequities in the provision of care in the public versus private sector, with the private sector having grown three thousand-fold over two decades, despite only serving a minority of the population [7]. Many patients in the public sector with end-stage kidney disease (ESKD) do not have access to dialysis [8]. Strategies to delay progression of renal failure is therefore an important goal.

2.2.2 Diagnosis of CKD

Kidney failure is usually diagnosed when serum urea and creatinine levels are raised. The duration of the impaired function is important to differentiate between acute, chronic or acute on chronic kidney disease. Once a diagnosis of CKD is made, it is important to stage the disease. There are five different stages of kidney disease which are classified by the glomerular filtration rate (GFR) and albuminuria as shown in Table 2.1, with stage five being ESKD where renal replacement therapies are needed for survival [9].

Table 2.1 Chronic kidney disease stages according to GFR and albuminuria

GFR grade	GFR ml/min. 1.73 m^2	Interpretation of kidney function	Persistent albuminuria category	ACR (mg/kg)	Interpretation of ACR
1	> 90	Normal	1	< 30	Normal to mildly increased
2	60-89	Mildly decreased	2	30-300	Moderately increased
3a	45-59	Mildly to moderately decreased	3	>300	Severely increased
3b	30-44	Moderately to severely decreased			
4	15-29	Severely decreased			
5	<15	Kidney failure			

[1]

ACR: Albumin : creatinine ratio; GFR: Glomerular filtration rate

2.2.3 Pathophysiology and complications of CKD

As kidney disease progresses, there is a noticeable decline in kidney function; serum urea and creatinine levels steadily increase. With deterioration to stage four, the ability to concentrate and excrete urine, potassium and phosphate is progressively lost [1]. As patients approach stage five, they develop uraemic symptoms that include nausea, vomiting, anorexia, weight loss, malaise and weakness [1].

Complications of CKD include the following:

- Hypertension

Hypertension is one of the most common complications of CKD, and uncontrolled hypertension can accelerate the progression of kidney and cardiovascular disease and increase mortality [10]. Hypertension in CKD is also affected by the kidney's decreased ability to remove salt; salt sensitivity is important to treat in CKD [11]. Both detection of hypertension and control of blood pressure are suboptimal in CKD patients and should be addressed to improve outcomes [10].

- CVD

CVD is the main cause of mortality in CKD patients [12]. Mortality from CVD increases as CKD progresses. CVD is the main cause of death in CKD patients and has a mortality rate 30 times higher in CKD [5]. Although the causes of the high prevalence are related to atherosclerotic factors, there is a higher risk associated with non-atherosclerotic factors such as left ventricular hypertrophy, valvular disease and vascular calcification [10]. These result in heart failure, arrhythmias and sudden death. Therefore, only controlling for atherosclerotic factors such as blood pressure and high cholesterol will not be sufficient to prevent CVD events. The complications of CKD should be treated in addition to traditional cardiovascular risk factors to reduce the high rates of mortality associated with the disease [10].

- CKD-bone mineral disease

CKD-bone mineral disorders are related to the high levels of serum phosphate, impaired calcium absorption, inactivation of vitamin D and high serum parathyroid hormone (PTH) that result from kidney failure [10]. They are mainly due to the hyperphosphatemia that sets off a series of metabolic events, resulting in high PTH which cause metabolic bone disease [7]. The Kidney Disease: Improving Global

Outcomes (KDIGO) [13] recently developed guidelines for the diagnosis and treatment of CKD-bone mineral disease. These encompass the treatment of abnormal phosphate and (PTH), renal osteodystrophy, soft tissue calcification and treatment of other biochemical abnormalities. However, the true benefits of these treatments in interventions are unproven, as many pre-clinical data of developments in this area have not translated into practical applications [13].

- Anaemia

The aetiology of anaemia in CKD is multifactorial. Anaemia develops in CKD owing to the inability of the kidney to produce erythropoietin (EPO) which induces erythropoiesis [14]. Other factors include uraemic-induced inhibitors of erythropoiesis, reduced erythrocyte turnover, altered iron metabolism and the increased need for iron due to EPO therapy [15]. Many CKD patients have a true iron deficiency as well as functional iron deficiency.

An excess of hepcidin in CKD also contributes to impaired iron mobilisation and dietary absorption [15]. The mechanism involves inflammatory cytokines and the degradation of the iron exporter ferroportin [15]. Cytokines enhance hepcidin transcription, most likely as a mechanism to sequester iron from invading pathogens. Hepcidin binds to and degrades ferroportin in duodenal enterocytes, macrophages and hepatocytes to prevent the entry of iron into the plasma, leading to anaemia [16].

EPO is generally effective in the treatment of anaemia but is also associated with CVD, cancer risks and other complications [10]. Iron therapy is used in the treatment of anaemia; however the role of iron therapy in the presence of high ferritin levels which are common in CKD, is controversial [15]. Treatment should be directed at underlying pathologies causing the anaemia.

- Metabolic acidosis

Metabolic acidosis is common in CKD and occurs when the acid intake and generation exceeds the excretion [10]. This could be caused by the increased production of volatile acids, increased loss of bicarbonate and decreased excretion of acid [17]. As the number of functioning nephrons progressively declines in CKD, acid excretion is initially maintained by increased ammonia excretion per nephron; however as the GFR continues to decline, there is a decrease in the amount of ammonia excreted [18]. The acidosis results in many complications, including increased protein breakdown, bone

disease, muscle wasting related to insulin resistance, inflammation, hypotension, malaise and worsening of secondary hyperparathyroidism [10, 17].

- Sodium and water retention

As CKD advances to stage four and five, there is loss of sodium balance. Sodium and water retention is common and leads to oedema, which increases blood pressure [19]. This further aggravates CVD, mainly in the form of left ventricular hypertrophy [10]. The measures for treatment of blood pressure include antihypertensive medication, diuretics and dietary sodium restriction [20].

- Appetite and inflammation

Anorexia manifests as early as stage 3 CKD and is caused by hormonal dysregulation, including increased anorexigenic hormones [21]. Additional factors contributing to the reduced appetite include a build-up of waste products and taste changes [22], and inflammatory cytokines and alterations in appetite regulation such as altered amino acid metabolism. This increases the transfer of tryptophan across the blood–brain barrier and has a hyper-serotonergic effect. [21].

- Constipation

Constipation is a common complication of CKD, with a high prevalence [23]. Nephrologists do not consider it a serious condition since it does not have significant outcomes such as cardiovascular disease [23]. However, it may cause discomfort and affect the quality of life of patients. In CKD, it is caused by multiple factors including a low dietary fibre intake due to dietary restrictions, reduced gut motility due to gut dysbiosis, side effects of medications (potassium-lowering resins, phosphate binders and antihypertensives), decreased physical activity and co-morbid conditions affecting bowel movement such as hypertension and diabetes [24]. Treatment for constipation includes laxatives, prebiotics, probiotics, and synbiotics, as well as lifestyle factors such as an increased fibre and water intake and increased physical activity [24].

2.3 Nutritional status in CKD

Patients with CKD have high rates of malnutrition in terms of undernutrition, also known as protein–energy wasting in CKD, with reported prevalence of 20–65% [25–27]. The malnutrition associated with CKD is related to decreased body stores of protein and energy and is often associated with a reduced muscle mass [28]. This is mainly due to

many side effects related to the disease, with inflammatory cytokines being the main driver of malnutrition, in addition to reduced dietary intake related to the symptoms [21]. Many other factors also contribute to protein–energy wasting [28].

Assessing malnutrition is complex in CKD since there is no single measure that can predict malnutrition owing to the multiple metabolic functions that are affected. Instead, the International Society of Renal Nutrition and Metabolism (ISRNM) [28] formulated a set of criteria that need to be met to diagnose malnutrition specifically in CKD. These four criteria include the assessment of weight, muscle mass, dietary protein intake and biochemical markers. At least three of the four criteria need to be met for a diagnosis of malnutrition. The malnutrition inflammation score is another tool used to assess nutritional status in CKD. It has ten categories, seven of which comprise the subjective global assessment (SGA) score, while the other three are non-SGA categories including serum albumin, total iron binding capacity and body mass index (BMI) [29]. The total score gives an indication of the extent of malnutrition, with a higher score being associated with the severity of malnutrition. The seven-point subjective global assessment tool (SGA) [28] has also been used to describe nutritional status; however it has not been found to be diagnostic of nutritional status, but rather as a clinical marker. The SGA assesses recent dietary intake, history of weight loss, presence of gastrointestinal symptoms and oedema, and the extent of muscle and fat wasting, with the criteria scores ranging from well-nourished to severely malnourished [30]. These tools give a more comprehensive indication of the nutritional status. However, studies are not consistent in their use of the criteria, thus making comparisons difficult. These criteria are however specific to undernutrition and are not sensitive to obesity. The recent Kidney Disease Outcomes Quality Initiative™ (KDOQI) guidelines [31] indicate that there is limited evidence to suggest the use of one tool over another in identifying malnutrition. They suggest that individual components of nutritional assessment be used such as anthropometry, biochemistry, history of dietary intake, appetite issues and clinical signs. This should be done at least annually or when referred for a nutrition-related problem. Their guidelines do however recommend the use of the SGA and malnutrition inflammation score in stage 5 CKD [31]. However, these recommendations were mainly based on studies done on haemodialysis patients. An assessment tool incorporating criteria for the assessment of over and undernutrition in predialysis patients would be one to consider in future research.

High rates of obesity have recently been reported in CKD, with some studies showing a high prevalence in CKD of up to 65% [32, 33]. This may be due to the increasing prevalence of obesity globally and in South Africa, where as many as 68% of women and 31% of men are obese [34]. Obesity is associated with many chronic disease conditions such as diabetes, hypertension, CVD and CKD. These disorders often co-exist, especially if there is increased visceral fat and it forms part of the metabolic syndrome. This increased visceral fat increases the risk of CKD [12]. Obesity has not received significant attention in the past, owing to the concept of reverse epidemiology known as the obesity paradox. It has been reported in the literature that having a higher BMI is protective in CKD and reduces mortality [35–37]. However, this observation is controversial. Most of the benefits associated with obesity and improved outcomes have been in the haemodialysis population [36]. A meta-analysis of Ahmadi et al. [37] on the obesity paradox found varying results at different stages of CKD, with only three of the ten studies showing increased mortality with underweight patients, while a higher BMI showed a lower, no association or a higher association with mortality. More robust epidemiological studies suggest that obesity has a negative outcome in CKD [12]. A higher rate of obesity has been associated with a progressively increased risk of stages 4 and 5 CKD [38]. It may be that the higher BMI associated with improved outcomes in the obesity paradox is rather a reflection of the disease condition of the patient; more ill patients have a lower BMI owing to cachexia associated with the chronic disease and therefore have a poorer prognosis [12, 39]. Some of the patients included in one of the obesity paradox studies did not have CKD but rather an acute course of the disease, which may have influenced the results [12]. It has been suggested that the apparent obesity paradox is the result of selection bias or other methodological errors [12]. Age has not been discussed in any of the articles relating to the obesity paradox in CKD; most of the CKD population reported in studies are older and are more prone to sarcopenia which may result in higher mortality. Younger, obese CKD patients may have different outcomes in terms of risk and survival. Obesity should still be viewed as a risk in the CKD population and interventions should aim to reduce weight with careful monitoring.

2.4 Treatment of CKD

The main aim of conservative management in CKD is to delay the progression of the disease, thereby avoiding dialysis [1]. The treatment of the disease involves various interventions including lifestyle modifications and pharmacological treatments to

manage the many complications of the disease [20]. These interventions will improve survival, reduce cardiovascular events and ultimately improve the quality of life [20]. Figure 2.1 shows the various interventions that delay the progression of the disease.

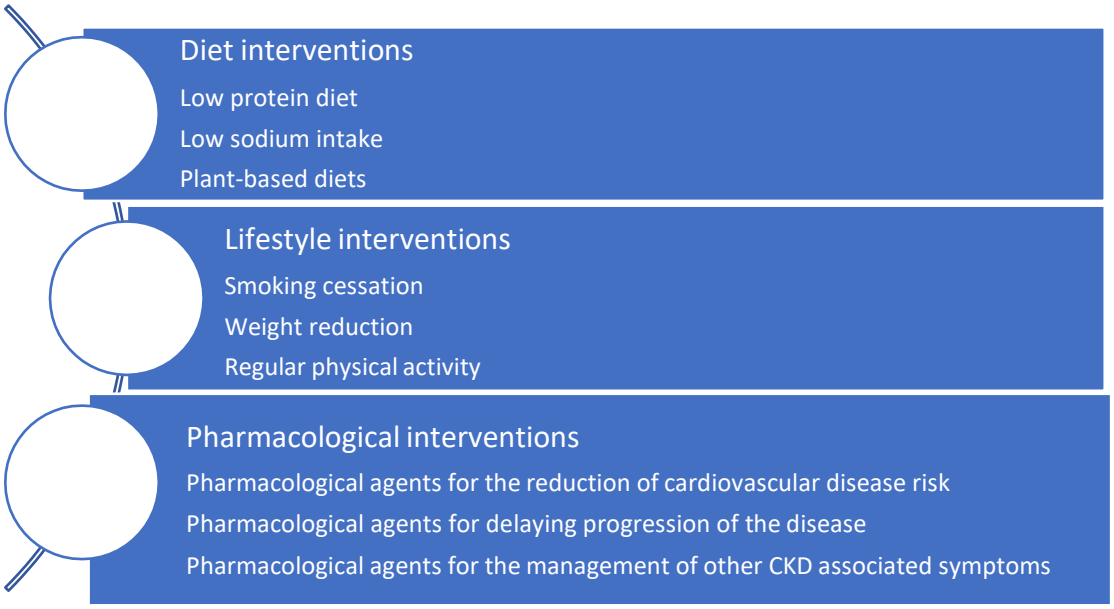


Figure 2.1 Intervention strategies in CKD to delay progression [20]

2.4.1 Medical management

The treatment of CKD is dependent on the stage of the disease and the symptoms experienced. In stages 1 to 3, treatment of the underlying conditions such as diabetes, CVD and hypertension and meeting target goals are important [1]. Table 2.2 briefly describes the disease conditions that need to be managed, the goals of treatment, the medical treatment involved and how this affects dietary prescriptions for the underlying conditions and advanced CKD. The medications prescribed should be appropriate for those with kidney dysfunction.

Table 2.2 Overview of treatments in CKD

Disease condition	Goals	Medical Treatment	Main nutrients affected in dietary prescription
CVD	Reduction of cardiovascular risk	Statins and anti-hypertensive medications	Sugar and fat intake
Hypertension	Control of blood pressure of 140/80mmHg or below 130/80mmHg	Anti-hypertensive medications	Salt intake

	with a high ACR		
Diabetes	Control of blood glucose levels Target HBA1c of 7–8%	Diabetes medication	Sugar, carbohydrate and fat intake
Advanced CKD (Stage four and five)			
Anaemia	Manage anaemia	EPO therapy Iron and vitamin supplements	Protein, vitamin and mineral intake
Electrolyte and acid base balance disturbances	Control electrolyte imbalances	Phosphate binder, vitamin D and calcium oral bicarbonate supplementation	Salt, potassium, calcium and phosphate intake

[1] ACR: albumin: creatinine ratio; EPO: erythropoietin; CVD: cardiovascular disease, CKD: chronic kidney disease

It is only in stage 4 to 5 that the kidney is not able to adequately conserve fluid and electrolytes and that more stringent measures are needed to control symptoms. Referral to a nephrologist should be done at GFR less than 30 ml/min/1.73 m^2 or albuminuria ≥300 mg per 24 hours [1]. There are pharmacological agents recommended for delaying the progression of CKD in those with diabetes and membranous nephropathy, although this falls outside the scope of this review.

2.4.2 Other lifestyle modifications

- Smoking cessation

Smoking cessation has been recommended for all patients with CKD due to the increased risk of incident CKD and all-cause mortality [20].

- Physical activity
- The effect of regular physical activity on kidney outcomes in predialysis patients is mainly based on observational studies, with studies showing improved kidney function and reduced blood pressure [20].

2.4.3 Dietary management

2.4.3.1. Nutritional requirements in predialysis CKD

Nutritional requirements in CKD are complex, since many nutrients need to be adapted to compensate for the metabolic derangements of the disease. Initial nutrition recommendations were developed by KDOQI by multidisciplinary working groups to

provide consensus practice standards based on evidence, opinion or a combination of both [40]. KDIGO was established in 2003, to provide global evidence-based guidelines in CKD [9]. These guidelines were older and mainly based on expert opinion with limited clinical intervention trials [31].

The requirements for individual nutrients are discussed according to the KDOQI/KDIGO recommendations for predialysis patients only. These recommendations have recently been updated [31]. The discussion will focus on the older guidelines and how these have been updated. Most of the dietary recommendations and education to patients in the study have been based on the old guidelines, since the newer guidelines were published very recently. Table 2.3 summarizes the nutritional requirements in predialysis patients.

Table 2.3 Nutritional requirements in predialysis CKD

Nutrients (unit)	Older guidelines [40][9]	Updated guidelines [31]
Energy (kcal/kg)	30–35	25–35
Total protein (g/kg)	0.6–0.8	0.55–0.6
Plant protein	50% of protein intake	No recommendations
Animal protein	50% of protein intake	No recommendations
Total fat	25-35% of energy	No recommendations
Saturated fat	< 10 % of energy	No recommendations
Polyunsaturated fat	< 10% of energy	Omega 3:2g for lowering triglycerides
Carbohydrates	50–60% of energy	No recommendations
Calcium (mg)	1 000–1 200	800–1000
Iron (mg)	10–18	No recommendations
Phosphate (mg)	800–1 000	Adjust according to blood values
Sodium (mg)	2 400	<2300
Potassium (mg)	2 000–3 000	Adjust according to blood values
Vitamin B6 (mg)	5	Supplement in poor intake or deficiencies
Folate (mg)	1 000	Supplement in poor intake of deficiencies
Vitamin D (mg)	5–10	Supplement in deficiencies
Vitamin A and K	Vitamin A not recommended Vitamin K individualised	Not recommended
Vitamin C (mg)	60–100	75 females 90 males

According to Table 2.3, energy requirements were set high at 30–35 kcal/kg due to the increased needs of the disease and to compensate for malnutrition [40]. The updated guidelines have reduced the lower limit to 25 kcal/per kg which should be more appropriate for obese individuals [31].

Protein requirements have also been controversial in predialysis CKD in patients. Very high protein intakes are discouraged since they accelerate renal disease progression as well as increase mortality [41]. Most research supports the recommendation of 0.8 g/kg protein to prevent malnutrition [42]. A meta-analysis by Rhee et al. [43] found that diets in CKD patients with protein < 0.8 g/kg compared to > 0.8 g/kg leads to higher serum bicarbonate levels, lower phosphorus levels, lower azotaemia, reduced CKD progression and a trend for reduced mortality. The updated guidelines have reduced this further to 0.55–0.6 g/kg; however in those with diabetes the recommendation is still 0.6–0.8 g/kg [31]. It is often difficult for patients to consume sufficient energy when restricting protein. In addition, uraemic symptoms may affect dietary intake and can lead to malnutrition [21]. There is also evidence of a reduction in disease progression with a very low protein intake together with keto-analogues [44]. Keto-analogues of amino acids, converted into amino acids via transamination processes in the body, may help improve nutritional deficiencies caused by diets restricted in protein [45]. However, the practicalities of a low-protein diet together with the high cost of the keto-analogues and low-protein foods need consideration, especially in a low-income country, where unemployment is high and food security is low.

Carbohydrate and fat requirements in CKD should be within the normal parameters of a general healthy diet. Owing to the increased risk of CVD in CKD, there should be a preference for unsaturated fat over saturated fat and trans fats, limiting salt, sugar, total fat and dietary cholesterol intake as well as increasing fibre intake [46]. The only fat recommendation the updated guidelines refer to are omega 3 fatty acids in the diet and suggest that 2 g/day be supplemented to lower serum triglyceride levels [31].

Regarding electrolytes and minerals, potassium intake is restricted; however, the evidence to support this is weak, since dietary potassium intake has a negligible effect on plasma potassium, with many other factors contributing to a high plasma potassium [47]. A slightly lower than normal requirement is recommended at 2 000– 4 000 mg per day [48]. The updated guidelines suggest prescribing potassium intake according to blood values. Potassium is widely found in foods, but is particularly high in milk, fruits and vegetables.

Sodium intake must be restricted since it influences blood pressure, fluid retention and worsens kidney function [41]. Sodium intake restriction of 2 400 mg a day [48] has been reduced to less than 2 300 mg [31].

Phosphate intake has been limited to 800–1000 mg/day due to the inability of the kidney to excrete it [48]. High serum phosphate and low serum calcium and vitamin D levels increase the risk of metabolic bone disease, hence the importance of balancing these nutrients. The bioavailability of phosphate differs in foods, with the most highly bioavailable foods being phosphorus food additives found in processed foods, high-phosphate meats, eggs and cheese, and plant phosphate being the least bioavailable due to the human intestine lacking the enzyme phytase to hydrolyse the phosphate. Therefore, although some plant products are high in phosphate, it is not a concern due to its limited bioavailability. It is important not to restrict too many plant products, including wholegrains, since they are an important source of fibre, minerals and vitamins. The updated guidelines therefore suggest prescribing phosphate intake based on blood values [31].

Fibre intake in CKD patients has been traditionally low, owing to the dietary restrictions. There is no specific requirement for fibre in CKD, but the prudent guideline of 25–30 g/day should suffice [49]. Plant-based diets high in fibre have been shown to favourably affect the gut microbiome [50]. There is evidence that dietary fibre affects the progression of chronic kidney renal disease [51–53]; however there are very limited studies investigating fibre and its effect on the gut microbiome in CKD patients and how this affects CKD progression.

Vitamins, particularly B vitamins and vitamin C, are important to consider in CKD, owing to reduced dietary intake of nutrients and losses due to symptoms of the disease at least to 100% of recommended daily values [40]. Dietary recommendations are given in Table 2.3. The updated guidelines only recommend supplementing intake if intake is reduced or if a deficiency is found [31].

2.4.3.2 Dietary adherence

Dietary modifications are important in the management of CKD, and if instituted early can delay the progression to ESKD [54]. However, dietary adherence is often low due to many restrictions on protein, fluid and electrolytes as previously discussed; the lack of autonomy; not knowing what to eat, and unpalatable diets; with up to 60% of patients not adhering to the dietary guidelines [55]. Low dietary adherence can affect the nutritional status of CKD individuals owing to inflammation, malnutrition and gut dysbiosis [56].

A diet that allows more variety with fewer restrictions would also improve dietary adherence. The evidence for the traditional CKD guidelines has been weak; it has mostly been based on clinical experience and limited clinical trials [57]. Therefore, many studies have advocated for the change to less restrictive diets in CKD based on the evidence and to improve adherence [47, 58, 59].

2.4.3.3 Dietary patterns

Dietary pattern studies in CKD show a reduction in mortality and disease progression with diets rich in wholegrains, fruits and vegetables compared with a Western diet [60–62]. Diet studies such as the Dietary Approaches to Stop Hypertension (DASH), the OmniHeart heart trial and the PREDIMED trial are all beneficial in reducing blood pressure and other CVD risk factors [57]. Although these diets may be more beneficial in early stages of CKD due to the high potassium and phosphorous composition, certain elements of these diets may be applied in end-stage CKD with careful planning. Gutiérrez et al. [63] showed in a study of nearly 4000 CKD participants that an American Southern diet pattern high in fried and processed foods was independently associated with mortality, whereas diets rich in fruits and vegetables were associated with a 23% reduction in mortality. The updated KDOQI guidelines have also recommended that the Mediterranean diet may improve lipid levels in stage 1 to 5 of CKD and that increased fruit and vegetable intake may improve weight, blood pressure and net acid production [31]. Diaz-Lopez et al. [60] showed a reduction in GFR with a Mediterranean diet, but not albumin : creatinine ratio in PREDIMED trial participants with a high CKD risk. Plant-based diets have also shown a reduction in GFR decline, improvement of acidosis and cardiovascular profiles, and a decline in mortality [20, 64]. Stanford et al. [65] demonstrated how a healthy plant-based diet was associated with improved uraemic toxins and gut microbiota. However, most of the plant-based studies are observational or experimental; there is a lack of randomised controlled trials showing these effects [20].

The focus of dietary education should be on the diet as a whole and not only individual nutrients [62].

2.5 Healthy gut microbiome

The gut is home to trillions of micro-organisms of over 1 000 species [66]. The gut microbiota accounts for 65% of the weight of faecal material, and the number of microorganisms in the gut far exceeds the number of cells in the body [67]. Each

individual has his or her own unique microbiota obtained after birth and influenced by the type of birth delivery, breastfeeding, diet, age, use of antibiotics, and environment [68]. The microbiome encompasses the entire micro-habitat, including the microorganisms (bacteria, fungi, viruses and protozoa), their genomes, and the surrounding environment [69]. The gut microbiota receives its nutrition from ingested dietary components (carbohydrates, proteins and fat) and host components such as mucus and shed epithelial cells [70]. During the process of using these nutrients, energy is produced for normal cellular processes. Protein fermentation produces toxic compounds such as phenols and indoles which may be harmful to the host in high amounts, whereas carbohydrate fermentation produces short-chain fatty acids (SCFAs) which are beneficial to the host [71]. The gut microbiome is therefore responsible for maintaining normal gut integrity and promoting immunological functions, and is involved with nutrient uptake and metabolism, degradation of oxalates and preventing the proliferation of harmful microorganisms [56]. These important functions have resulted in the gut microbiome being considered a functional ‘organ’ [72].

Disruption of the normal functioning of the gut microbiota has been associated with many chronic diseases, including diabetes, immunological disorders, CVD, autism, depression, and obesity [56, 68]. Recent studies have implicated gut dysbiosis as being a key factor in the progression of CKD and its complications [56, 73].

The most predominant phyla in the gut are the *Firmicutes*, *Bacteroidetes* and *Proteobacteria*; with *Actinobacteria* phylum comprising a smaller percentage of bacteria [70]. The class, order, family and genus that make up these phyla are shown in Figure 2.2.

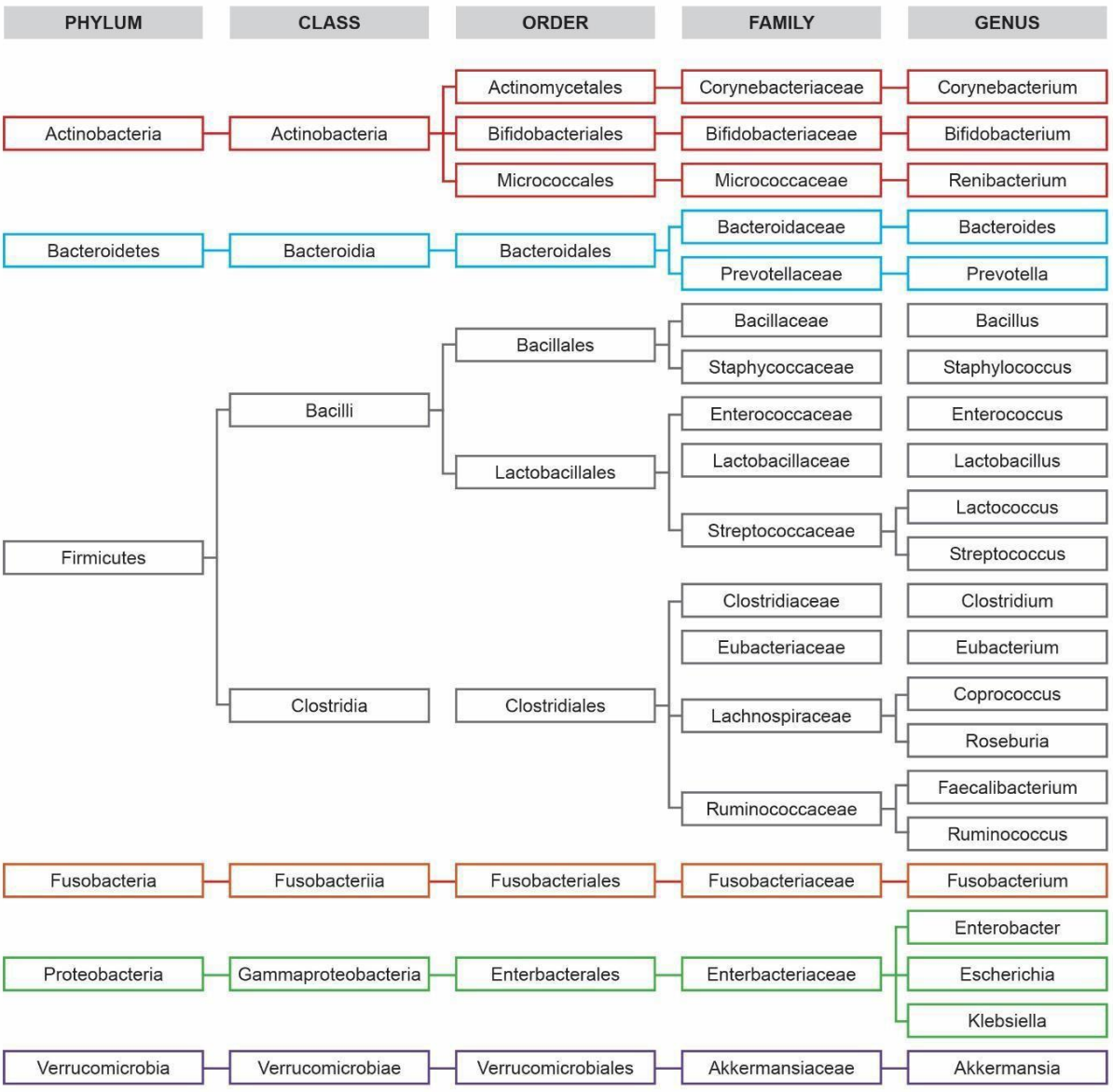


Figure 2.2 Phylogenetic tree of taxa [23]

*Used with permission of the authors

Bacteroidetes and *Firmicutes* are the most abundant phyla in the gut. *Firmicutes* consist of large numbers, comprising over 200 genera, including *Lactobacillus*, *Enterococcus*, *Ruminococcus* and *Clostridium* [23]. *Bacteroidetes* include bacteria from Bacteroides and *Prevotella* species [70]. *Bacteroidetes* has a positive effect on health, whereas most of the bacteria from the *Firmicutes* phyla have a less positive effect. Keeping a good ratio between the two is important to ensure a healthy gut microbiome. There is speculation that obese individuals have an imbalance in this ratio [68, 74]. *Actinobacteria* are less abundant and mainly represented by the species *Bifidobacterium* [23].

The distribution of the phyla varies across populations. Studies of pooled metagenomic data from healthy individuals in Europe, Japan and North America show that broad patterns of gut microbiota could be identified, irrespective of the country of origin [70, 75]. These are referred to as enterotypes. Three enterotypes have been identified in the literature, based on the abundance of three species: firstly *Bacteroides*, secondly *Prevotella* and thirdly *Ruminococcus* [75]. However, more recently Vandeputte et al. [76] identified four enterotypes: *Bacteroides* 1, *Bacteroides* 2, *Prevotella* and *Ruminococcus* from study- and disease-based cohort samples, complemented with samples from the Flemish Gut Microflora project. These four enterotypes have also been reported by others [77, 78]. *Bacteroides* 2 specifically has been associated with inflammatory markers, stool moisture and chronic disease conditions such as inflammatory bowel disease [76]. Vieira-Silva et al. [79] explored obesity-associated microbiota alterations in the MetaCardis Body Mass Index Spectrum cohort (*n*=888). They reported a higher level of obesity and inflammation associated with the *Bacteroides* 2 enterotype in a sub-cohort of patients not treated with statins. They also observed that gut dysbiosis was negatively correlated with statin therapy; those on statins had a lower prevalence of the *Bacteroides* 2 enterotype. The *Bacteroides* 2 enterotype is therefore associated with a dysbiotic microbiome composition.

Enterotyping is performed using Dirichlet Multinomial Mixtures (DMM) [76]. This is a probabilistic modelling of microbiome data. A frequency matrix represents the number of times each taxon is observed in each sample. Vectors are produced and describe communities by the taxa probabilities. The vectors are produced from the Dirichlet mixture components and observed samples are produced through the multinomial mixtures. The mixture components then cluster communities into distinct enterotypes of similar composition [80].

2.5.1 Gut microbiota analysis

There have been many recent advancements and achievements in gut microbiome research, with new sequencing technologies and data analysis being developed [81]. Ninety-eight percent of species in the gut have been assigned by 16s ribosomal RNA (16s rRNA) techniques to be part of four phyla: *Firmicutes* (64%), *Bacteroidetes* (24%), *Proteobacteria* (8%) and *Actinobacteria* (3%) [82].

DNA is easy to extract and sequence allowing for the development of different methods of high-throughput sequencing (HTs). The most used methods include amplicon

sequencing and metagenomic sequencing. Amplicon sequencing focuses on specific gene markers and not the whole genome, whereas metagenomic sequencing focuses on a culture-independent genomic analysis of microbes from the environment, using a shotgun metagenome sequencing approach [83]. 16S rRNA amplicon sequencing is the most common method used. The information obtained this way can only reach genus-level resolution. Metagenomic sequencing provides more detailed genomic information and taxonomic resolution than amplicon sequencing methods [84]. It is a much more expensive technique. Figure 2.3 illustrates the amplicon method.

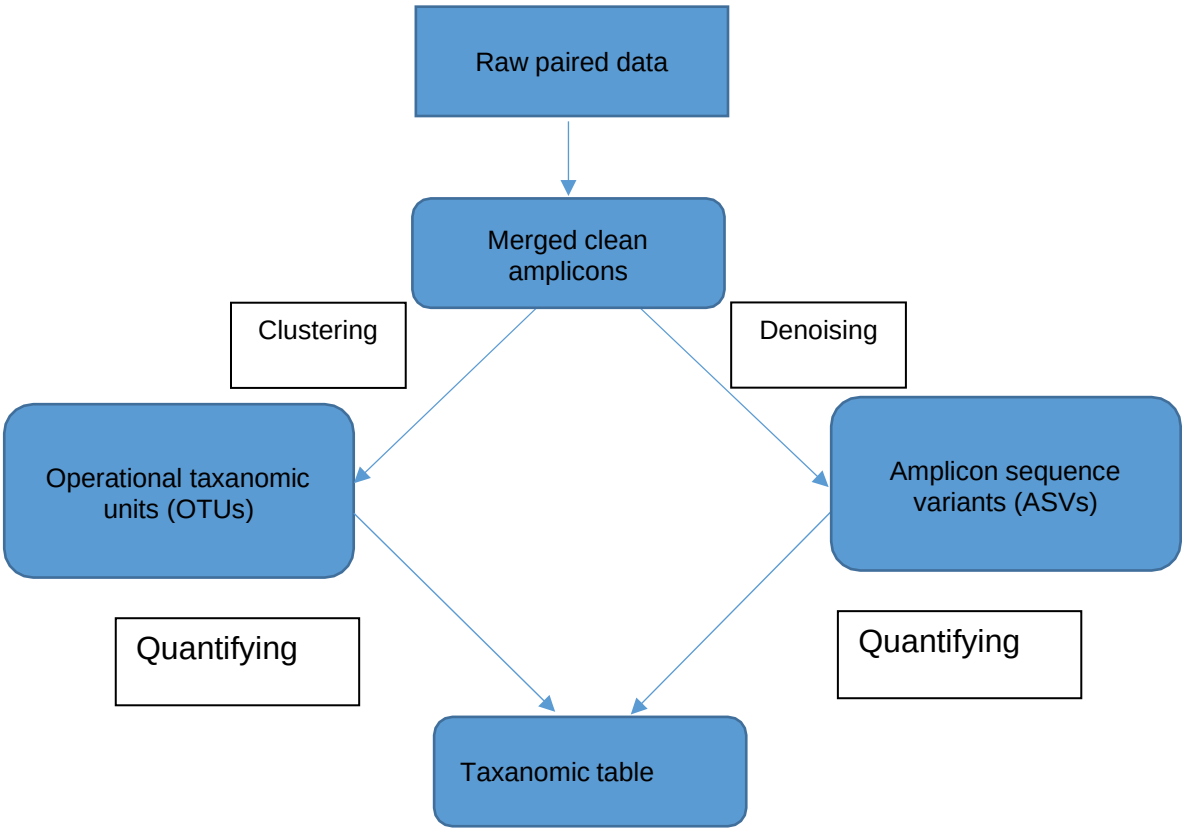


Figure 2.3 Amplicon sequencing methods [84]

With amplicon sequencing as described by Liu et al., the raw reads are converted into a feature table; they are usually in paired end-bases mode and generated from Illumina platforms [84]. Then raw amplicon data are grouped based on their barcode sequences (demultiplexing). Next the paired reads are merged to form amplicon sequences; in this process the barcode primers are also removed. Low-quality amplicons are usually removed as part of quality control. All of these steps can be completed using USEARCH or QIME Pipelines [84]. Pipelines is a program or script that combines several or even dozens of software programs in a certain way to allow for complex analysis of information. The next step is to select representative sequences as proxies

for species. There are two major approaches to doing this: firstly, clustering into operational taxonomic units (OTUs) using UPARSE algorithm clusters, and secondly, the DADA2 algorithm for denoising into amplicon sequence variants (ASVs) [84]. The similarity index for OTUs is 97%; that means that marker-gene sequences have 97% similarity. The drawback of this is it may miss subtle and real biological sequence variations [81]. Denoising has recently been developed to compensate for this variation; it detects the error profiles to resolve data into exact sequence features. From here, it is quantified and a feature table is obtained. At the same time, the sequences can be assigned taxonomically into kingdom, phylum, class, order and species levels. Commonly used databases for 16s sequence classification include SILVA, Greengenes and the Ribosomal Database Project [85]. Further pipelines can be used to convert this into functional information since the amplicon sequencing only detects taxonomic composition [81]. The OTUs are often described in terms of their diversity. Diversity is an important concept in reporting OTUs, since many disease conditions are characterised by low microbial diversity of certain organisms. OTUs can be described by alpha diversity (relative abundance), which is the number (richness) and distribution (evenness) of taxa within a single sample, whereas beta diversity measures the absolute overlap and examines how many taxa are shared between samples [86].

There is only one trial to our knowledge investigating the effect of a synbiotic on the gut microbiome and uraemic toxins in CKD patients who used 16S rRNA amplicon sequencing methods to produce the taxonomic profiles and diversity tables with QIIME software [87]. This study showed a significant increase in Bifidobacterium species in the synbiotic group. Stanford et al. [50] used the 16S rRNA technique to sequence data using QIIME software to assess the associations among plant-based quality, uraemic toxins and gut microbiota profiles in adults undergoing haemodialysis. They found the quality of the diet and food selections either supressed or promoted certain microbes which may have influenced the levels or gut-derived uraemic toxins. More recently, a study investigated the gut microbiome-related effects of berberine and probiotics on type 2 diabetes using shotgun metagenomic sequencing. This study's methods were able to identify a microbial-related mechanism underlying the antidiabetic effect of berberine and a probiotic [88]. These studies demonstrate that the16S rRNA techniques offer basic taxonomic information, whereas the shotgun metagenomic sequencing offers functional information.

2.5.2 Effect of dietary factors on microbial diversity

Research has shown that diet affects the gut microbiome in different ways [71, 89]. Metagenomic studies have shown that the gut microbiome is influenced by the ability of microbes to metabolise simple sugars, showing that the microbiota must adapt to the nutrients available in the small intestine. Animal and plant-based diets also influence the composition of the gut microbiome. *Prevotella* has been associated with diets high in fibre and polysaccharides, whereas *Bacteroides* in associated with high-fat, animal-based Western diets [90]. Figure 2.4 shows the phyla associated with increased and decreased bacterial diversity associated with dietary factors and the specific genera associated with the production of short-chain fatty acids. Plant-based diets increase faecal microbial diversity and encourage the growth of bacteria that produce SCFAs, whereas animal-based diets have the opposite effect and are considered 'bad' for gut health [91].

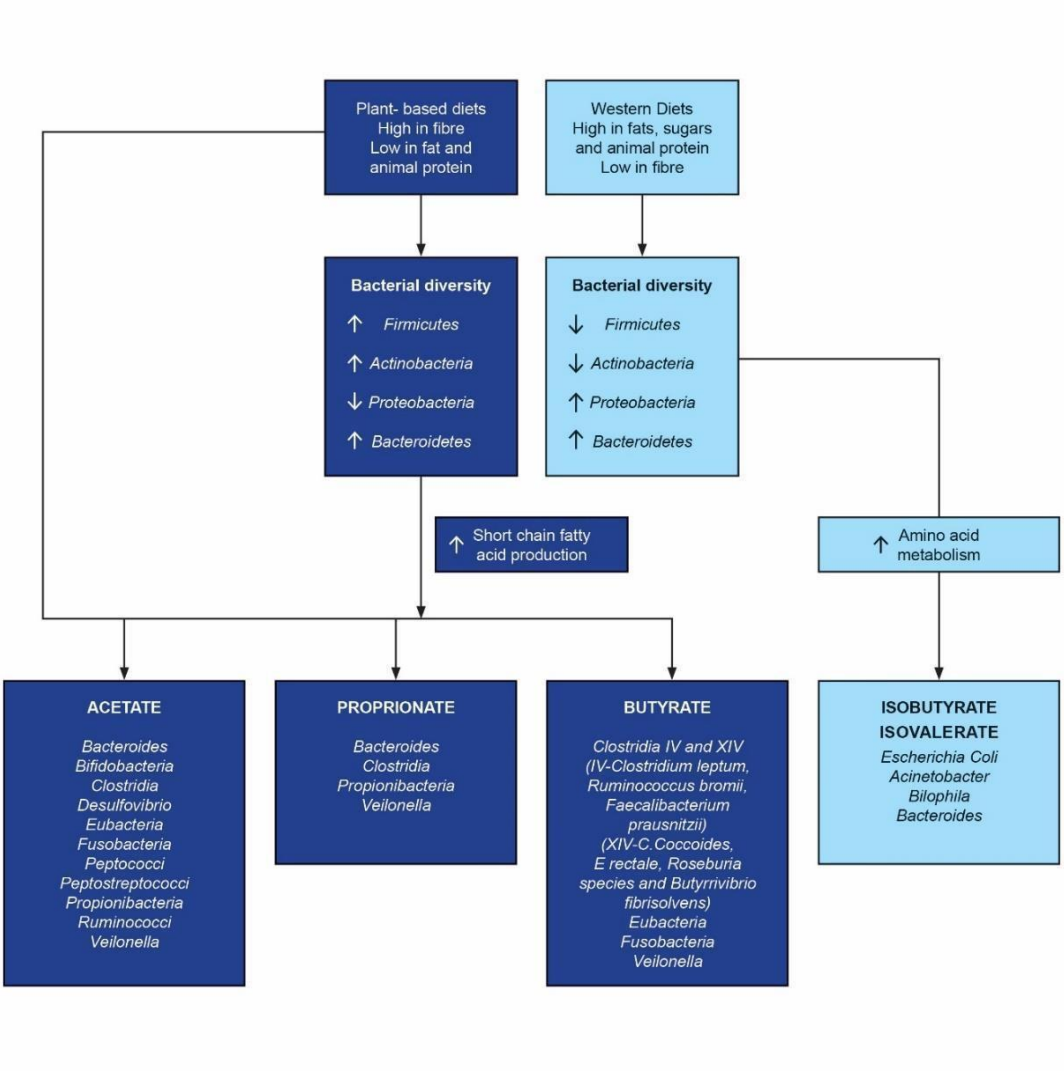


Figure 2.4 Dietary factors and microbiota involved in the production of the various short-chain fatty acids [70, 91]

2.5.2.1 Effect of protein on microbial diversity

There are distinct differences between the effect of plant and animal protein on microbial diversity in healthy subjects. Animal protein is known to increase bile-tolerant anaerobes such as *Bacteroides*, *Alistipes*, and *Bilophila*, while decreasing *Roseburia*, *Eubacterium*, and *Ruminococcus* [92, 93]. Plant protein, such as pea and whey protein, has been shown to increase gut-commensal *Lactobacillus* and *Bifidobacteria*, while whey protein additionally decreases *Bacteroides fragilis* and *Clostridium perfringens* [92].

2.5.2.2 Effect of dietary fat on microbial diversity

Studies on the effect of fat on the gut microbiome in healthy subjects report that a high- fat diet and saturated fat increase pathogenic bacteria such as *Clostridium*, *Bacteroides*, *Bilophila* and *Faecalibacterium prausnitzii* [93], while a low-fat diet and a high unsaturated fat diet increase lactic acid bacteria and *Bifidobacteria* [92, 93]. High fat diets have also been associated with an increased *Firmicutes : Bacteroides* ratio [94].

2.5.2.3 Effect of dietary carbohydrates on microbial diversity.

Saccharolytic bacteria result in the production of SCFAs which are beneficial to the host. Approximately 20–60 g of carbohydrates reach the colon daily, where complex carbohydrates are converted to oligosaccharides and monosaccharides, and then fermented into carbon dioxide, hydrogen, ethanol, and SCFAs [23]. The major SCFAs produced by carbohydrate fermentation include butyrate, acetate, and propionate [56]. Acetate and propionate are absorbed into portal circulation and metabolised by the liver [23]. While a portion of acetate is metabolised in other tissues, including adipose tissues, propionate is mostly metabolised by the liver and lowers cholesterol and blood glucose, while butyrate is the major energy source for the epithelial cells [23]. SCFAs have many functions, including maintaining tight junction integrity in the colon and increased epithelial repair in response to injury, as well as to allow for cell differentiation [73]. There are many microbiota involved in the colonic fermentation of carbohydrates into SCFAs as shown in Figure 2.4.

The gut epithelium acts as a protective defence mechanism barrier and consists of a dense inner layer and outer looser mucus layer which is penetrable to bacteria; these layers serve many functions and act to lubricate the alimentary tract, allowing for the passage of stools [23, 89]. Mucin-producing microbial species may also increase when

fibre is deficient in the gut [23]. These species also contribute to shaping the gut microbiota [89].

Dietary fibre and resistant starches increase bacterial abundance and gene richness of gut-commensal bacteria, especially *Lactobacilli* and *Bifidobacteria*, *Roseburia*, *Eubacteria* and *Ruminococcus*, while reducing pathogenic *Clostridia* and *Enterococcus* [92]. Long-term dietary pattern studies show carbohydrate-rich diets favour *Prevotella* abundances; in addition, high-fibre plant-based diets showed a higher *Prevotella : Bacteroides* ratio [94]

These findings emphasise the importance of healthy eating guidelines such as low-fat, high-fibre and plant-based proteins, not only for cardiovascular benefit and general health, but for improving microbial diversity.

2.5.2.4 Effect of obesity on gut microbial diversity

Al-Asal et al. [68] reported in a recent review that obese subjects have less microbial diversity than those of normal weight, with an increased *Firmicutes : Bacteroidetes* ratio. However, this was not confirmed in a meta-analysis of seven studies that failed to find a lower proportion of *Bacteroidetes* in obese individuals compared with normal weight individuals after analysing the relative abundance of sequences at the phyla level. They did, however, find fewer *Firmicutes*, *Bifidobacteria* and *Methanobrevibacter* [82]. In a review by Zheng et al., some studies showed that caloric restriction causes a reduction in *Bacteroidetes* over *Firmicutes*, while others showed an increase in *Bacteroidetes*, thereby reducing the *Firmicutes : Bacteroidetes* ratio [95]. These inconsistent results may be due to relative changes in the lower-level taxa without affecting the relative abundance of the major phyla [74].

In a very recent review, it was concluded that the *Firmicutes : Bacteroides* ratio cannot be used as hallmark of obesity owing to the many controversies surrounding it. The authors cited reasons such as methodological flaws in DNA sequencing, poor characterisation of participants and failure to take into account lifestyle factors that affect the gut microbiome diversity [96]. Further research is needed on obesity to clarify these controversies.

2.6 Gut dysbiosis in CKD

The relationship between the gut microbiome and CKD has been termed the “kidney-gut axis” as seen in Figure 2.5, which is caused by a vicious cycle contributing to the

associated CKD complications. Gut dysbiosis refers to an ill-defined state of alterations in the function and composition of gut microbiota due to a host microbial imbalance, with decreased diversity and low-grade inflammation at the gut mucosal barrier [98]. There are various causes in CKD for gut dysbiosis as illustrated in Figure 2.5 below.

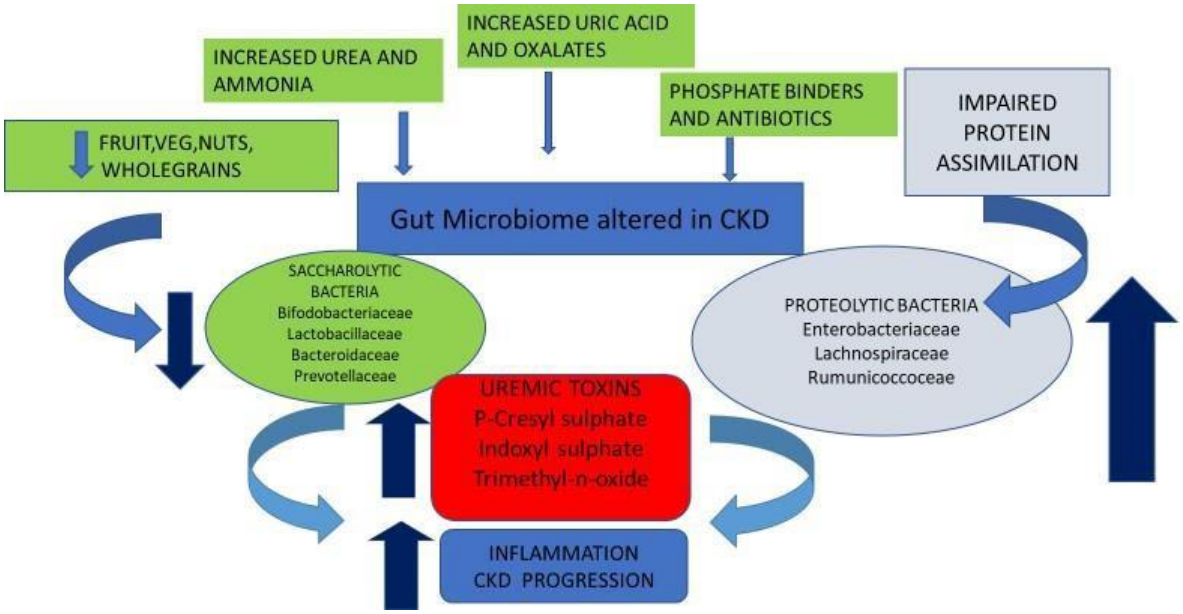


Figure 2.5 Gut dysbiosis in CKD [67, 73, 97]

The composition of the gut flora is influenced by the biochemical environment. There are four main reasons for the biochemical imbalance. Firstly, it is mainly due to inability of the kidneys to excrete waste products, so that the colon becomes the main excretory organ to maintain body homeostasis; urea is excreted into the gut and the end-product of urea metabolism is ammonia. This increases luminal pH due to the action of urease-producing bacteria and causes changes in the gut barrier [72]. This damage is mainly caused by the disruption of the tight junctions in the intestinal epithelia that normally maintain a seal between adjacent epithelial cells. Secondly, there is an increase in gut uric acid and oxalates, since these are excreted owing to renal function decline [67]. Thirdly, the low intake of dietary fibre and other dietary restrictions due to CKD lead to a change in the function and bacterial composition of the gut, as described above [56, 67]. Fourthly, the use of phosphate binders and antibiotics also has an impact on the gut microbiome composition [67]. Additionally, the link between constipation and its

contribution to gut dysbiosis in CKD has recently been explored and is thought to contribute to gut dysbiosis in various ways [23].

Two types of fermentation occur in the gut, saccharolytic and proteolytic. CKD dietary restrictions limit fibre and antioxidant-rich foods, since these foods are high in potassium and phosphates. These recommendations favour proteolytic fermentation as shown in Figure 2.5, which results in excessive uraemic toxin production and reduces saccharolytic production of SCFAs from carbohydrates [90]. Prolonged colonic transit time reduces the available carbohydrates for fermentation, reducing the production of SCFAs [70].

There are many factors that affect the availability of protein in the gut. Protein assimilation is affected by the amount of protein consumed, whether it is cooked or uncooked, whether it is animal or plant protein, as well as the presence of other dietary components such as resistant starch [67]. These factors affect the absorption of protein, with up to 35% of protein being unabsorbed in the healthy population [67]. This is aggravated in CKD and is affected by various factors such as gastric suppression treatment, uraemic pancreopathy, gastrointestinal motility disorders, and small bowel bacterial overgrowth [91]. The end products of protein fermentation include ammonia, hydrogen, carbon dioxide, methane, amines, phenols and indoles, to name a few; some of these are precursors of uraemic toxins and known to be pathogenic to the host in high concentrations [23, 73]. High levels of protein intake have also been correlated with high levels of *p*-cresyl sulfate (*p*CS), confirming that protein assimilation efficiency worsens as kidney function declines [91].

Other studies have identified other factors contributing to the disruption of the tight junctions, including intestinal wall oedema, metabolic acidosis, and slow colonic transit time [99]. These biochemical changes in the gut microbiota generate uraemic precursor toxin metabolites as shown in Figure 2.5, which further worsen kidney function. The evidence for the presence of these toxins in CKD has been shown in a study comparing plasma solutes in haemodialysis in patients who had intact colons with those who had a total colectomy [100]. Through high-resolution mass spectrometry, researchers found over 30 different individual features present in haemodialysis patients with intact colons that were either absent or at low concentrations compared with those without colons. These features were higher in the plasma of dialysis patients with intact colons compared with healthy controls, suggesting that they represented uraemic solutes.

They verified the colonic origin of uraemic toxins such as indoxyl sulfate (IxS) and *p*CS, as well as a few other uraemic toxins.

The disruption of the tight epithelial junctions affects the translocation of endotoxins and other microbial components across the intestinal barrier due to increased permeability, resulting in systemic inflammation [56]. The intestinal epithelial cells will express inflammatory cytokines which increase the Th1 and Th17 response by dendritic cells and macrophages, producing increased IgG by B-cells [101]. The gut's saccharolytic microbiota are essential as fuel cells for the colonic epithelial cells and regulatory T-lymphocytes. These cells are already reduced in renal failure, and this, together with a low-fibre diet and the above-mentioned factors, accounts for CKD-associated systemic inflammation [67].

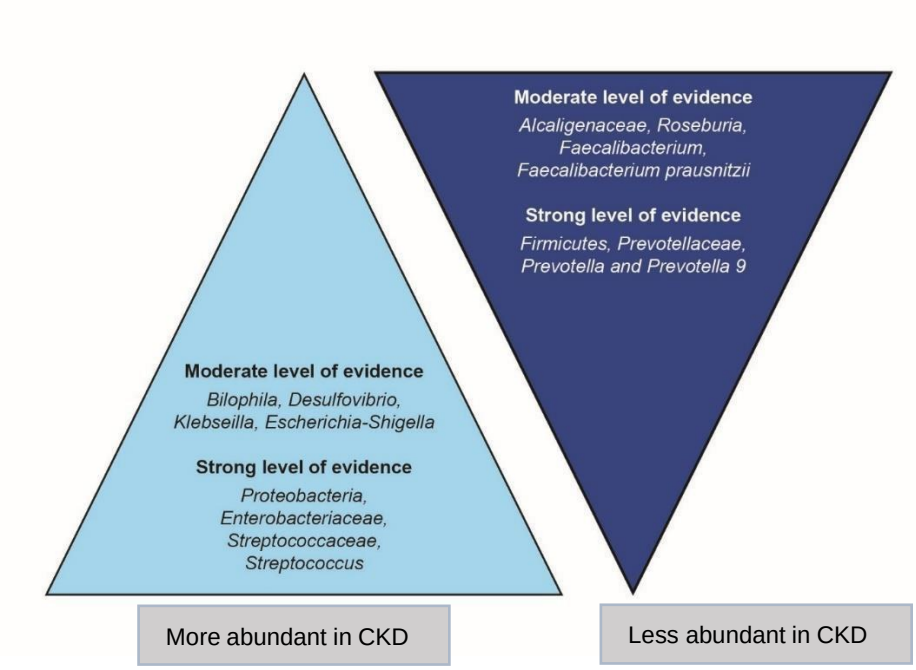
2.6.1 Effect of gut dysbiosis on neuroendocrine pathways in CKD

Gut dysbiosis has recently been found to influence neurohormonal pathways in the establishment and progression of kidney failure. Jazani et al. [102] describe in their review how the gut microbiota influences the neuroendocrine pathway through the activation of the hypothalamic–pituitary–adrenal axis, induction of hormone release, production of neurotransmitters, altered tryptophan metabolism, and through changes in the vagus nerve, thus promoting CKD progression.

2.7 Gut microbial changes in CKD

The gut microbiota in CKD are different from those in healthy controls. Vaziri et al. [103] demonstrated a significant difference in abundance in 190 taxonomic units in CKD. There were lower numbers of normal colonic bacteria such as *Lactobacilli* and *Prevotellaceae* families and 100 times higher numbers of *Enterobacteria* and *Enterococci* species which are usually present in smaller numbers [103]. Sampaio-Maia et al. [73] illustrate in their review of various studies investigating gut microbiota in CKD that the predominant bacteria that are reduced in CKD are from the *Lactobacillaceae*, *Bifidobacteriaceae* (especially the genus *Bifidobacterium*), *Prevotellaceae* and *Bacteroidaceae* families; whereas those that are increased are from the Enterobacteriaceae family (especially the genera *Escherichia*, *Enterobacter*, and *Klebsiella* species). All the above results corroborate the findings of Vaziri et al. [103] in nephrectomised rats; they isolated the effect of uraemia on the gut microbiome from inter-individual differences, co-morbidities, dietary and pharmacological interventions, and found most notably decreases in *Lactobacillaceae* and

Prevotellaceae families. Another study specifically elucidated 19 microbial families that were dominant in CKD, 12 families possessing urease, five possessing uricase and three containing *p*CS and IxS enzymes. The four families that were depleted included two containing SCFAs enzymes [104]. Jiang et al. [105] evaluated gut microbiome differences in their Chinese population in CKD patients compared with healthy controls, using 16S rRNA sequencing. Eleven bacterial taxa were overrepresented in CKD samples and 22 taxa were overrepresented in healthy controls. They found reduced levels of butyrate-producing bacteria such as *Roseburia*, *Faecalibacterium*, *Clostridium*, *Coprococcus* and *Prevotella*. In addition, *Roseburia, Faecalibacterium prausnitzii, Prevotella* and universal bacteria were negatively correlated with CRP and cystatin C. Mazidi et al. [106] and Li et al. [107] also showed similar findings in relation to gut microbiome differences and correlations; in addition they found *Akkermansia* to be positively correlated with production of interleukin 10, suggesting it to be a possible therapeutic target to attenuate the progression of inflammation .



A recent systematic review by Stanford et al. [65] characterised the gut microbiota composition in adults with kidney disease compared with healthy controls. They elaborated on organisms that are characteristic of CKD, based on strong and moderate levels of evidence as shown in Figure 2.6, most of which corroborate the above findings.

Figure 2.6 Gut microbiota abundances based on levels of evidence in CKD [65]

Levels of evidence as defined by the article. Strong level of evidence: consistent findings (<75%) in at least two high-quality studies. Moderate level of evidence: consistent findings (>75%) in one high-quality study and at least one low- quality study.

2.8 Uraemic toxins

The uraemic syndrome results from an increased retention of solutes that would normally be excreted by the kidney. These solutes are referred to as toxins when they negatively interact with biologic functions [108]. The toxins are divided into three groups based on their molecular weight (MW), small (MW<500da), medium (MW>500da) and protein-bound solutes (≤500da) [109]. The protein-bound solutes are known to express toxic effects in a direct or indirect way, but it is the free concentrations and not the protein-bound form that exert the toxic effect [110]. The extent of protein binding depends on various factors such as solute concentration, protein concentration, solute affinity concentration and the absence of competing solutes [110]. Uraemic toxins bind to specific proteins in variable degrees. Structural changes in proteins can also affect binding. It is therefore hypothesised that the degree of protein binding changes as the disease progresses, due to post-translational modifications of proteins which include oxidation, carbamylation, and glycosylation. Low molecular weight toxins then bind to these modified proteins with high affinity and increase the overall molecular weight of the modified protein, which then escapes from glomerular filtration [110]. The concentration of these proteins increases and contributes to the development of CVD [111].

The gut-derived uraemic toxins that are increased in CKD include urea, IxS, *p*CS, *p*-glucuronide (pCG), indoxyl acetic acid (IAA) and trimethylamine-N-oxide(TMNO), which all have been shown to be pro-inflammatory, accelerate renal disease progression and increase CVD risk and mortality [112–114]. Koppe et al. [115] further elucidate the impact of toxins generated by the intestinal microbiome: these include insulin resistance, reactive oxygen species formation, and endothelial dysfunction. In CKD there is decreased absorption of amino acids in the small intestine; this, together with a prolonged transit time, increases the concentration of amino acids in the colon which increases the expansion of proteolytic species and the metabolization of aromatic amino acids into precursors of uraemic toxins [101].

Urea is a small uraemic toxin solute that is produced by the liver but undergoes further metabolism in the body and the gastrointestinal tract [109]. The intermediate end-product of protein metabolism is ammonia, which is then turned into urea via the ornithine cycle [56, 109]. Urea is a waste product since it cannot be further metabolised and must be excreted in the urine. Urea can also pass into the gastrointestinal tract

where urease-producing organisms cleave the urea into ammonia and carbon dioxide, producing amino acids which can be utilised for normal processes in the body [56]. This process changes the intestinal pH which shapes the microbial communities of the gut [103]. There are many urease-producing organisms [103].

Intestinal bacteria break down tryptophan to indole, which is metabolised to indole-3-acetic acid and then to IxS in the liver [101]. Indole-producing bacteria include *Escherichia coli* and *Clostridium sporogenes* [116]. Tryptophanase activity occurs mostly in the distal colon. However, in CKD, owing to diets being low in fibre, there is a reduction in SCFA activity and the activity of the tryptophanase occurs along the whole length of the colon [85].

These toxins originate in the colon, where aromatic amino acids are metabolised by bacteria into phenolic (*p*-cresol and phenol) and indolic compounds (indole and IAA). *p*-Cresol is detoxified in the mucosal cells and the liver, where it is sulfated into *p*CS and IxS, and glucuronidated into pCG [101]. *p*CS is formed by the anaerobic bacterial fermentation of tyrosine by the following organisms: *Clostridium difficile*, *Faecalibacterium prausnitzii*, *Subdoligranulum* and selected strains of *Bifidobacterium and Lactobacillus* [116, 117].

IAA is produced from tryptophan metabolism by bacteria and from tryptophan in other tissues [101]. IAA is toxic and enhances cell peroxidase activity and is involved with glucose and glutamine metabolism activation.

TMNO is formed from bacterial fermentation of carnitine and lecithin that releases trimethylamine which is converted to TMNO by monooxygenase enzymes [118]. Animal-based diets tend to increase TMNO levels.

Sulphur-containing dietary products can also produce hydrogen sulphide (H_2S), which can be toxic at high levels.

The intermediate breakdown products involved in these processes are transported via the portal vein to the liver where they are further metabolised. These intermediate products, namely, indole and indole-3-acetic acid enhance mucosal cell-barrier function, whereas *p*-cresyl, H_2S and TMO intermediates are pro-inflammatory. The end-products *p*CS, IxS, TMNO and H_2S enter circulation, where some bind to albumin. They are eventually excreted in the urine. The exact effect of each intermediate and end product on intestinal and immunological factors is shown in Table 2.4, and has been clearly explained in a recent review by Glorieux et al. [101]. Additionally, features

of how they are bound to proteins and levels compared with normal values are shown in Table 2.4.

The uraemic toxins affect nearly every organ system in the body. The toxins are only reduced by dialysis but to a limited degree owing to their high protein-binding capacity; other strategies to reduce levels such as modulating the gut microbiota are needed in the predialysis population [115]. These toxins reversibly bind to albumin; however it is only the free fraction that is removed by dialysis [119].

Table 2.4 Uraemic toxin features and physiological effects

Uraemic toxin	Features	Physiological effects
Indoxyl sulfate (IxS)	90–98% of indole is bound to plasma proteins. IxS levels increase 40-fold in uraemia compared with normal healthy individuals	Oxidative stress Accelerates inflammatory state Activation of the renin-angiotensin system Accelerated aortic calcification Bone and mineral disorders
p-Cresyl sulfate (*p*CS)	*p*CS is bound to protein at about 95%, although to a lesser degree than IS. *p*CS levels increase to 2-fold in uraemia more than in normal healthy individuals.	Renal tubular damage Increased inflammation Reduced antioxidant capacity Activation of the renin angiotensin system Increased insulin resistance CVD risk All-cause and CVD mortality
p-Cresyl glucuronide (*p*CG)	*p*CG is bound to protein at about 10%.	Increased reactive oxygen species (ROS) Impaired blood flow
Trimethyl-N-oxide (TMNO)	TMNO is not bound to proteins and is cleared through tubular secretion.	Accelerated atherosclerosis Accelerated kidney disease progression
Indoxyl acetic acid (IAA)	IAA increase to 5 times the normal values and is bound to protein at about 92%.	Oxidative stress Accelerated inflammatory state

[85, 101, 108, 113, 114]

2.9 Clinical studies on the effect of diet and supplements on kidney function, uraemic toxins and the gut microbiome

The microbial changes at different stages of CKD are not well established; however for both predialysis and haemodialysis patients, there are distinct differences in the colonic microbiome compared with healthy volunteers [103, 120]. Poesen et al. [120] showed that although there were distinct differences between the gut microbiome and healthy individuals, the microbiome of CKD compared with matched healthy household

contacts showed no clear differences; this suggests that the diet and environmental factors may outweigh the importance of a reduced GFR .

Interventions that are targeted at modulating the gut microbiome in CKD include synbiotics, prebiotics and probiotics [56]. Probiotics are bacteria that are beneficial to intestinal health; they are defined as organisms that when administered in adequate amounts confer health benefits [56]. There are several strains of these bacteria that favourably affect the gut, including *Lactobacilli*, *Bifidobacteria* and *Streptococci* species. Probiotic studies have shown varying results in the reduction of uraemic toxins and inflammatory markers, depending on the strain used. A meta-analysis by Koppe et al. [121] found limited benefits in CKD with the use of probiotics and described how inflammation potentially can be increased by the hydrolysis of urea . Studies also report that by simply adding the organisms into the gut with a prebiotic to change the biochemical milieu of the microbiome may be futile [63,116]. Synbiotics implies co-administration of pre- and probiotics. Studies investigating synbiotics show mixed results.

Investigating prebiotics may be more promising in altering the gut microbiome as discussed below.

2.10 Prebiotics

Prebiotics are non-digestible food ingredients that promote the proliferation of healthy bacteria. Prebiotics include fibre, although not all fibres are prebiotic. A prebiotic has the following characteristics: it is resistant to gastric acid, can be hydrolysed by enzymes and absorbed in the upper gastrointestinal tract, can be fermented by intestinal microflora and increases the growth of intestinal microbiota [122]. It includes fructo-oligosaccharides, galacto-oligosaccharides, and lactulose. However, many other compounds have been found to have prebiotic potential and the definition of a prebiotic has been challenged. Bindels et al. [123] define prebiotics focusing on the characteristics of the microbiota, including their diversity and the production of SCFAs that are important for immunity and gastrointestinal function. This adds more compounds that can be classified as prebiotics, including resistant starches, pectin, arabinoxylan, ß-glucans, wholegrains and dietary fibre. However, uncertainties remain regarding the mechanism of action and categorisation of prebiotics [115].

Table 2.5 expands on the studies that investigated the effects of prebiotics, fibre and synbiotics in CKD patients, which are further discussed below.

Table 2.5 Effect of various prebiotics and dietary fibre on renal outcomes

	Study population	Fibre type	Follow - up weeks	Diet	Outcomes studied	Study findings
Prebiotics and dietary fibre						
Bliss et al., 1996 *n* =16 [51]	CKD	Gum arabic 50 g day	4	Low-protein diet	Urea Faecal bacterial mass and nitrogen content	Urea significantly reduced Faecal bacteria mass and nitrogen content significantly increased
Rampton, 1984 *n* = 9 [52]	CRF	Hemicelluloses: Arabinogalactan and ispaghula	5	Not indicated	Urea levels	Urea reduction 11% and 19% respectively
Younes et al., 2006 [53]	CKD patients	Fermentable carbohydrate 40 g enriched diet (powder, bread, snacks)	5	0.8 g protein for both control and intervention groups	Nitrogen excretion in the stool, stool weight Urea, creatinine, and creatinine clearance Nutritional parameters (weight, BMI)	Significant increase in nitrogen excretion in stool in the intervention group Significant reduction in urea No significant effect on creatinine and creatinine clearance Significant increase in weight in the intervention group
Ali, 2008 *n* = 36 [124]	Haemodialysis patients	Gum arabic 50 g	12	Not indicated	Urea, creatinine, phosphorous	Reduction in urea, creatinine and phosphorous
Elamin et al., 2017 *n* = 36 [125]	CKD	Gum arabic 10 g, 20 g, 40 g	4	Not indicated	Urea Creatinine IxS CRP and other markers	No change in urea, creatinine or IxS CRP reduced
Sirich et al., 2014 (*n* = 20 in each group) [126]	Haemodialysis patients	Resistant starch 15 g day	6	Not indicated	IxS *p*CS	Significant reduction in IxS, reduction in *p*CS, although not significant
Poesen et al., 2016 *n* = 39 [127]	CKD predialysis patients	Arabinoxylan oligosaccharides 20 g/day	4	Not indicated	Uraemic toxins Serum and 24hr urinary levels (*p*CS, IxS, TMNO, phenylacetylglutamine, insulin resistance	No significant findings
Meijers et al., 2009	Haemodialysis patients	Oligofructose inulin 20 g/day	4	Not indicated	*p*CS, IxS and its generation rates	*p*CS reduced by 20%

n = 22 [128]						
Tayebi et al., 2016 Khosroshahi (*n* = 32)[129]	CRF	Lactulose 30ml/day	8	Usual diet, no fermented products	Urea, creatinine, *Lactobacilli* and *Bifidobacteria*	Significant decrease in creatinine, significant increase in *Lactobacilli* and *Bifidobacteria*
Pender, 1989 n= 20 [130]	Haemodialysis patients	Unprocessed wheat bran	4	Not indicated	Potassium phosphate Bowel habit	Reduced potassium but not significant and significant increase in phosphate levels Improved bowel habit
Salmean, 2013 *n* = 15 [131]	CKD patients	23 g added fibre (pea hull, inulin and resistant corn dextrin-cereal, cookies, snack bars)	4	Usual diet	Stool frequency Quality of life and appetite scores, sleep scales, glucose, cholesterol	Significant increase in stool weight, cholesterol and TC:HDL ratio, significant decrease in KDQOL mental health component while physical component increased with no significant overall effect, sleep scale reduced significantly
Synbiotics						
Rossi et al., 2016 (*n* = 37)[132]	CKD patients	Synbiotic containing prebiotics inulin and oligosaccharides and probiotics including 9 different strains of *Lactobacillus, Bifidobacteria* and *Streptococcus*	6	Seen by dietitian to stabilise protein and fibre intakes	Uraemic toxins, IxS and *p*CS, urea, creatinine, GFR, inflammatory markers, oxidative stress markers, dietary intake, symptom scores and stool microbiota profile	A significant reduction in *p*CS, enrichment of *Bifidobacterium* and depletion of *ruminococcaceae*
Lopes et al., 2016 (*n* = 58) [133]	Haemodialysis patients	Synbiotic 40 g Sorghum with unfermented milk	7	Not indicated	*p*CS, IxS, urea, creatinine, SCFA markers and PH	Significant reduction in *p*CS, IxS and urea
Guida et al., 2014 (*n* = 30) [134]	CKD patients	Synbiotic containing inulin and tapioca starch and 9 different strains of *Lactobacillus*, *Bifidobacteria* and *Streptococcus*	4	No diet intervention	*p*CS and gastrointestinal (GIT) symptoms	Significant reduction in *p*CS, but no change in GIT symptoms

2.10.1 Effect of prebiotics on kidney function

A systematic review of the effects of prebiotics on kidney outcomes reports significant reductions in serum urea and creatinine [97]. However, the sample size of studies has been small with a lack of randomised controlled trials. A more recent meta-analysis however showed that prebiotics have a small effect on kidney function as measured by GFR, urea and creatinine [135].

Studies show reductions in serum urea levels with prebiotics such as gum arabic, [51, 124] hemicelluloses, and ispaghula [52] as the fibre source, while other studies show no change in urea with fermentable carbohydrates [53] and gum arabic [125].

2.10.2 Effect of prebiotics on uraemic toxins in CKD

Sirich et al. [126] report a reduction in the disruption of the tight epithelial junction, oxidative stress and uraemic toxins in 40 chronic haemodialysis patients on six weeks of 15 g of resistant starch over six weeks. The uraemic toxins that were significantly reduced were IxS; *p*CS was also reduced, although not significantly.

Conversely, some studies show no effect of prebiotics on uraemic toxins; a study using the prebiotic arabinoxylan oligosaccharides (AXOS) showed no reduction in serum and urinary uraemic toxins *p*CS, IxS and TMNO in predialysis patients [127]. This is also different from the effect AXOS has on healthy volunteers, where it was found to reduce urinary *p*CS levels [136]. Meijers et al. [128] found a reduction in *p*CS with 20 g of inulin in haemodialysis patients used for four weeks. In a meta-analysis by Wu et al., seven studies involving 203 CKD patients investigating the effect of fibre on uraemic toxins, a significant reduction in *p*CS, but not IxS was found [137], while a more recent meta-analysis showed a significant reduction in both *p*CS and IxS [138]. They found that the reduction in *p*CS was not influenced by dose, dialysis, diabetes or intervention time, while a significant reduction in IxS was found in dialysis patients compared with non-dialysis patients.

2.10.3 Effect of synbiotics on uraemic toxins

Rossi et al. [132] also showed a reduction in *p*CS using a synbiotic containing a 7.5 g powder of a mixture of inulin, fructo-oligosaccharides and galacto-oligosaccharides, and one capsule of different strains of the *Lactobacillus*, *Bifidobacteria* and *Streptococcus* species.

Guida et al. [134] also found a significant reduction in *p*CS with synbiotic use containing probiotics and a small amount of inulin and resistant starch in CKD predialysis participants, although the placebo also contained resistant starch. They did not investigate IxS. A study investigating the use of synbiotic meal, containing 40 g of sorghum as a prebiotic and 100 ml of an unfermented probiotic milk containing *Bifidobacterium longum* in 58 subjects over seven weeks, showed a significant reduction in IxS, *p*CS, pH levels and urea [133]. These results are very promising since they are the first to show significant reductions in both IxS and *p*CS levels, although in haemodialysis participants.

2.10.4 Effect of multiple strategies in lowering of IxS

From the available research, IxS seems to be more difficult to reduce. Leong et al. suggested that ways to reduce IxS would be to reduce its production through protein restriction, and reduce absorption though drug treatment or through colonic manipulation [113]. One study of protein restriction showed a 37% reduction of IxS at very low protein intakes of 0.3 g/kg with keto-analogues versus those with an intake of 0.6 g/kg [139]. Indoxyl is produced from tryptophan and therefore high intakes of protein increase these levels. The only studies that have reduced IxS involve resistant starch [126] and a synbiotic meal containing sorghum and probiotic milk [133].

2.11 Effects of synbiotics on the gut microbiome

There are not many studies that investigate the effect of synbiotics on the gut microbiota. Rossi et al. [132] showed a favourable modification of the gut microbiome with a significant increase in *Bifidobacteria* and a repletion of *Ruminococcaceae* with the use of a synbiotic as described above. The study samples were small with 17 individuals who received the intervention and 20 who received the placebo control over a period of six weeks. Another study investigating 30ml of lactulose administered for

eight weeks, found a significant increase in *Bifidobacteria* and *Lactobacilli* in CKD stage 3 and 4 patients [129].

It can be concluded from the research discussed that the following have a positive effect on *p*CS: oligosaccharides, and inulin in predialysis participants; in haemodialysis patients, there was a positive effect on *p*CS and IxS with a synbiotic meal containing sorghum and only in IxS with resistant starches; a positive effect was also reported on the gut microbiome with using a synbiotic containing oligosaccharides, inulin and probiotics.

2.12 Oat fibre effect on gut microbiome in non-CKD individuals

Oats has prebiotic properties due to it being a wholegrain; the β-glucan and the resistant starch present in oats have the most prominent effect on gut health [140]. Rose [140] indicated that wholegrains have the potential to change the gut microbiota even though they are poorly fermented. Oats has many different components which enhance the trophic effect on the gut microbiota. Both fermentable and non-fermentable fibres have bifidogenic properties.

Connolly et al. [141] investigated the prebiotic and cholesterol lowering effect of oats in “at- risk CVD” patients and found that 45 g of granola oats-based cereal increased the levels of *Bifidobacteria*, *Lactobacilli* and total bacteria, and reduced total cholesterol levels by 0.94 mmol /L and LDL levels by 0.4 mmol/L. This was at a dose of 1.3 g of β-glucan, which is nearly half of the amount recommended for cholesterol lowering. Maize has also shown significant improvements in increasing *Bifidobacteria* [142]. Valeur et al. [143] found that 60 g of oats improved gut microbial functions in healthy participants. In a recent study, also in healthy individuals, 3 g of β-glucan present in 100 g of pasta reduced the levels of *p*CS and improved endothelial vascular reactivity in healthy individuals [144]. The source of β-glucan was barley. In another study investigating the effects of six different oat products on three different individuals upon digestion, it was reported that all increased *Bifidobacterium* levels [145]. There were varying alterations of pH and metabolites, especially positive changes in lactate and butyrate, with a reduction in ammonia and SCFAs in the gut. Oat bran had the most bifidogenic effect. ß-glucan also showed a significant increase in *Bacteroides* and a moderate increase in *Prevotella* in individuals with elevated cholesterol levels without affecting bifidogenic properties, while many organisms correlated to CVD risk factors [146]. Studies also suggest that ß-glucan increase bile salt hydrolase activity (BSH), which in turn increase cholesterol excretion and bacteria in the gut with known BSH activity, such as

Bifidobacterium, *Bacteroides* and *Lactobaccillus* [147]. It therefore seems that β-glucans have many beneficial effects on lipids and the gut microbiome.

There are many characteristics of oats that make it a good option to study in CKD, including the presence of ß-glucan, its lipid characteristics, its high solubility, the presence of resistant starch and many phenolic compounds [140] as well as affecting bile salt metabolism [147] . β-glucans are non-polysaccharide branched chains of glucose polymers; each β-glucan has a different structure, solubility, molecular weight and viscosity depending on the source. These characteristics affect their functional properties. β-glucans have also shown prebiotic properties [141]. The processing of oats also affects the release of the β-glucans: mechanical processing releases the β-glucan, whereas hydrothermal processing reduces the extractability of the β-glucan and also increases its viscosity which can in turn reduce cholesterol and glucose absorption [145].

2.13 Conclusion

CKD prevalence is increasing. With limited resources and access to replacement treatment in developing countries, the aim should be to prevent the progression to end-stage CKD. CKD has many complications related to the disease which change the biochemical environment of the intestine and can cause a generation and translocation of products that can cause inflammation, malnutrition, and uraemic toxicity. Dietary modifications such as increasing prebiotic fibre intake and reducing protein intake may improve outcomes. These and other dietary factors, together with appropriate medical management, may reduce uraemic toxin levels and improve the gut microbiome dysbiosis that accompanies CKD. There are no studies on the effect of oats or β-glucans on the gut microbiome and uraemic toxins in CKD patients, which makes it a novel food to explore owing to its many beneficial properties.

2.14 References

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CHAPTER 3 : OVERVIEW OF METHODOLOGY

This chapter provides a general overview of the methodology used. More detailed information can be found in each relevant chapter.

3.1 Setting of the study

The study was conducted at Tygerberg Hospital Chronic Renal Outpatient Clinic, Cape Town, South Africa. Tygerberg serves a drainage area in the Western Cape with a population of over 3.4 million people. The drainage areas include Northern Metro sub-districts, Khayelitsha, north of Spine Road, Eastern Tygerberg, West Coast, Cape Winelands, Overberg, and rural districts. The hospital has 1 384 beds, and sees up to 107 215 inpatients and 492 670 outpatients a year [1].

3.2 Population

All CKD stage 3 to 5 pre-dialysis patients followed up at the outpatient CKD clinic at Tygerberg Hospital and living in Cape Town were eligible for inclusion.

3.3 Sample size calculation, inclusion and exclusion criteria

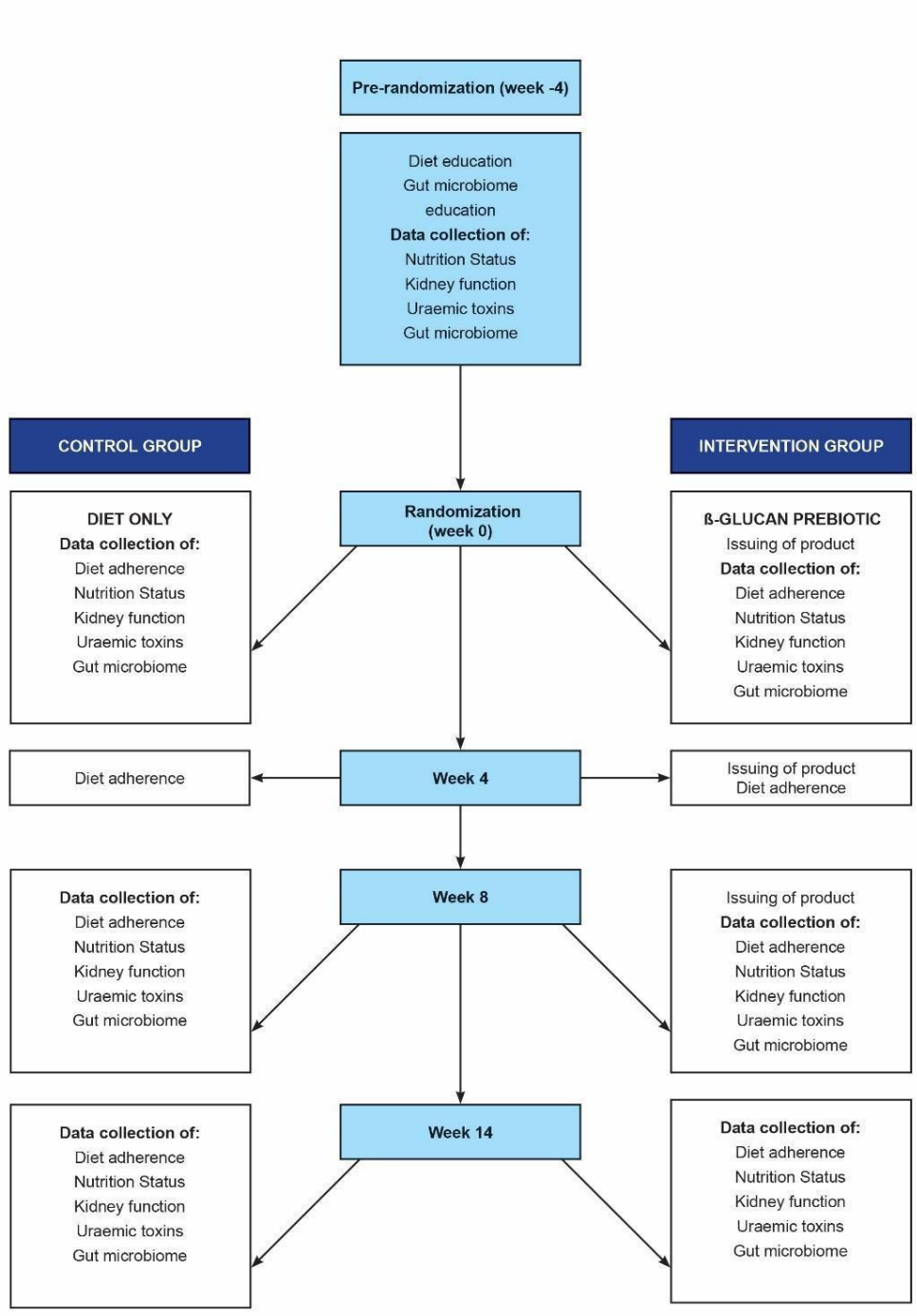
The sample size was calculated to detect a reduction in urea (12%), creatinine (5%) and an increase in *Lactobacillus* of 20% using a two-sample *t*-test using the Power Analysis Sample Size software (PASS program) based on previous study outcomes [2]. A power of 90% was used. The minimum amounts needed to detect a change were 16 for urea, 15 for creatinine and 23 for *Lactobaccillus* in each arm of the trial. Thirty-five participants were enrolled in the intervention group and thirty-five in the control group owing to expected dropouts. The inclusion criteria included participants over 18 years old and who were in stage 3 to 5 CKD (<60ml/min/1.73 m^2). The exclusion criteria included: taking antibiotics, prebiotics or probiotic supplements currently or in the past four weeks, patients with inflammatory bowel disease, bowel malignancy, previous colorectal surgery (or any other serious bowel disorder), pregnancy, diabetes mellitus, coeliac disease, human immunodeficiency virus (HIV) disease, malignant hypertension, crescentic glomerular nephritis, patients on immunosuppressant medications, and those expected to start immediate dialysis.

3.4 Sample selection

Participants were attending the clinic for their scheduled doctor appointments; their files were screened if their GFR was less 60 mL/min/1.73 m^2 to determine if they met the inclusion or exclusion criteria. All participants that met the inclusion criteria were recruited. They were approached to explain the study and invited to participate.

3.5 Methods overview

This study was a randomised controlled intervention study over 18 weeks (Pan African Clinical Registry trial number: PACTR202002892187265). There was a run-in diet period of four weeks where participants were counselled on CKD diet before being randomised. Participants were randomised using a simple computer-generated randomized list with an equal allocation ratio; ie. 1 for the intervention group (35 participants) and 2 (35 participants) for the control group. Sequentially numbered sealed opaque envelopes were used to assign group allocation. At randomisation, the intervention group received the ß-glucan supplement and continued the diet, while the control group continued with the diet only. A placebo was not used since we did not want to introduce another variable that could affect the gut microbiome. There were follow-ups at week 8 and week 14 after randomisation for all the measurements as shown in Figure 3.1, and a visit at week 4 for measuring dietary adherence and issuing of the supplement. Measurements were repeated at each follow-up visit as described overleaf.



Nutrition status includes anthropometry, dietary assessment, other nutrition-related biochemical tests

Figure 3.1: Study flow diagram

3.5.1 Pre-randomisation baseline procedure

The principal investigator (PI) or research assistant (RA) completed the consent form with the participant; thereafter the participant saw the PI or RA for assessment and dietary education.

3.5.2 Follow-up procedures

The participant saw the PI for assessment and RA for the issuing of the supplement and re-enforcing of the diet in the intervention group and the control group.

The reason for the PI only doing the assessments was not to bias the measurement results owing to her being single-blinded and not knowing to which group participants were assigned to. Participants could not be blinded since there was no placebo.

3.6 Randomisation procedure

This section is described in Chapter 7.

3.7 Data collection

Data collected included socio-demographic and medical information, anthropometry, biochemistry, clinical information, gastrointestinal (GIT) symptoms, Bristol Stool Scale score sheet, dietary intake assessment, and a diet-adherence score sheet. The methodology not explained in subsequent chapters is described here.

a. Socio-demographic and medical information

The following information was obtained at baseline from the medical file and the patient by the PI or RA:

- Age, gender, income, ethnicity, educational level and home language.
- Medical diagnosis and cause of renal failure
- Current medications

b. Anthropometry

Anthropometry was performed by the PI and/or RA at the following times: pre-randomisation (- 4 weeks), baseline visit at randomisation (8 weeks and 14 weeks). Standard operating procedures (SOPs) were used for anthropometry [3].

The analysis of the anthropometry is explained in Chapters 5 and 6.

- Weight

Weight was measured with a calibrated Seca scale (Seca GmbH, Hamburg, Germany) to the nearest 0.1 kg. Participants were minimally clothed and asked

to face forward when stepping onto the scale with feet slightly apart, distributing the weight evenly [3]. Weight was adjusted for oedema as described in the clinical section. An average of three measurements was taken.

- Height

Height was measured at baseline only with a stadiometer which was firmly placed against the wall, ensuring the stadiometer was level. Participants were asked to remove shoes and anything on top of the head. Participants were asked to hold their heads in the Frankfort horizontal plane and asked to inhale while the head plane was lowered to the vertex for the measurement to be taken to the nearest 0.1 mm. Participants were asked to exhale after this [3]. An average of three measurements was taken.

- Waist circumference

Waist circumference was measured in centimetres to the nearest mm using a standard non-stretchable tape measure while the participants stood upright with their arms relaxed to the side. The measurement was taken with a tape measure at the narrowest point of the waist or midway between the tenth rib and the ileac crest. The measurement was taken at the end of at least three completed breath expirations [3]. An average of three measurements was taken.

- Mid-upper arm circumference

Patients were relaxed with arms hanging by their sides. The circumference was taken with a tape measure at the level of the mid-acromial radial and perpendicular to the long axis of the arm [4]. An average of three measurements was taken.

- Triceps skinfold

The triceps skinfold were measured at the site of the mid-acromial radial. The site is located at the posterior aspect of the arm in the mid-line of the mid-acromial radial [4]. An average of three measurements was taken.

- Clinical assessment

Clinical assessment was performed by the PI and/or RA at the following times: pre-randomisation (- 4 weeks), baseline visit at randomisation (8 weeks and 14 weeks).

Participants were assessed for ankle oedema by applying pressure on the tibia of the ankle with thumb and releasing after five seconds. The investigator took note of the time that it took for the area to rebound; the longer it took to rebound the more severe the oedema was. The oedema was then categorised as mild (1 kg), moderate (5 kg) or severe (10 kg) and weights were adjusted according to the degree of oedema. Table 3.1 explains the indent measurement and the time it takes the skin to rebound to be able to make an accurate assessment.

Table 3.1 Weight adjustment according to the grading of oedema [5]

Grade	Indent measurement	Rebound time	Adjustment of actual weight
Mild	Barely detectable < 2 mm	Immediate rebound	-1 kg
Moderate	3–4 mm	Slight indentation < 15 s to rebound	-5kg
Severe	> 4mm	Deeper indentation > 15 s to rebound	-10kg

- Biochemistry

Serum samples were collected according to standard protocols and sent to the National Health Laboratories for further biochemical analysis at the following times: pre-randomisation (- 4 weeks), baseline visit at randomisation, 8 weeks and 14 weeks.

Biochemistry parameters such as urea, creatinine, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TG), C-reactive protein (CRP), phosphate, sodium and potassium were performed using standard kits in a fully automated Cobas C501 (Roche/Hitachi analyser, Germany- Manheim) using various enzymatic reactions as described in Table 3.1 below. Glomerular filtration rate was calculated by the Modification of Diet in Renal Disease (MDRD formula) as follows [6]:

eGFR mL/min/1.73 m^2 = 175 serum creatinine (mg/dL)$^{-1.154}$ x age$^{-0.203}$ x 0.742 (if

female. The calculation of LDL-C was calculated by using the traditional Friedewald's formula (F-LDL-C): F-LDL-C (mg/dl) = C-HDLC - TG/5 [7].

Table 3.2 Biochemical methods

Urea	This was a kinetic test with urease and glutamate dehydrogenase. Urea was hydrolysed by urease to form ammonia and carbonate. In the second reaction, L-glutamate was formed by the reaction of 2-oxoglutamate with ammonium in the presence of glutamate dehydrogenase and the co-enzyme nicotinamide adenine dinucleotide (NADH). The urea concentration was determined photometrically and was directly proportional to the rate of decrease in the NADH concentration.
Creatinine	This enzymatic test was based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to form glycine, formaldehyde and hydrogen peroxide. Catalysed by peroxidase, hydrogen peroxide that was liberated, reacted with 4-aminophenazone and 2,4,6-triiodo-3-hydrobenzoic acid to form a quinone imine chromogen. The creatinine concentration was directly proportional to the colour intensity of the quinone imine chromogen in the reaction mixture.
Cholesterol	This enzymatic colorimetric method involved the cleaving of cholesterol esters by cholesterol esterase to form free cholesterol and fatty acids. Cholesterol oxidase then catalysed the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide formed in the presence of peroxidase effected the oxidative coupling of phenol and 4-aminophenazone to form a red quinone-imine dye. The cholesterol concentration was directly

	proportional to the color intensity of the dye formed and measured by the increase in absorbance.
TG	This is an enzymatic colorimetric test. Triglycerides were hydrolysed by lipoprotein lipase to form glycerol and free fatty acids. Glycerol kinase then acted on glycerol to form glycerol-3-P, oxidised by glycerol phosphate oxidase to form hydrogen peroxide. Through the catalytic action of peroxidase, this reacted with chlorophenolic compound and 4-amino antipyrine to form a red coloured benzoquinone-monoimino-phenazone complex. The triglyceride concentration was directly proportional to the color intensity of the dye formed.
HDL	This was homogenous colorimetric test. In the presence of magnesium ions, dextran sulfate formed water soluble complexes with chylomicrons, LDL and very-low density lipoprotein (VLDL). These complexes were resistant to polyethylene glycol (PEG) enzymes. Cholesterol was broken down into free cholesterol and fatty acids by cholesterol esterase, which was oxidised by cholesterol oxidase to from cholestenone and hydrogen peroxide. The hydrogen peroxide generated reacted with 4-amino-antipyrine and sodium *N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HSDA) in the presence of peroxidase to form a purple blue dye. The HDL concentration was proportional to the intensity of the dye and measured phytometrically.
CRP	This test was a particle enhanced immunoturbidimetric assay. CRP agglutinated with latex particles coated with monoclonal anti-CRP antibodies. These aggregates were determined turbidimetrically.
Phosphate	This test used the molybdate ultraviolet method. In the presence of sulphuric acid, inorganic phosphate formed an ammonium phosphomolybdate complex with ammonium

	molybdate. The inorganic phosphate concentration is directly proportional to the phosphomolybdate concentration and is measured photometrically.
Sodium and potassium	The concentration of sodium and potassium is determined by ion sensitive electrodes (ISE). This test made use of the membrane materials to develop an electrical potential for the measurement of the ions. The system to determine the ion concentration involves the ISE, a reference electrode and electronic circuits to measure and test the electromotive force to give the test ion concentration.

[8]

3.8 Methods overview

The following methods are described in detail in chapters as indicated in the table below.

Table 3.3 Overview of the methods

Methods and analysis	Chapters
Dietary assessments	
Quantified food-frequency questionnaire (QFFQ) (Addendum B) The adaptation of the QFFQ to assess dietary intake and the analysis thereof are described in these chapters.	5, 6
Dietary adherence score sheet (Addendum D) The development of an adapted dietary adherence score sheet to assess adherence to the dietary education advised is described in this chapter.	6
Dietary compliance Dietary compliance is described in these chapters.	6, 7
Intervention	
Prebiotic information	7

The breakdown of the ß-glucan prebiotic is described in this chapter as well as instructions on its use.	
Diet education material and education The development of the dietary education material and detail of the dietary education are explained in these chapters.	4, 5
Gastrointestinal assessment/analysis	
Bristol Stool Scale Chart (Addendum E) The scoring of the Bristol Stool Scale Chart is described in this chapter.	7
Gastrointestinal symptoms	7
Gut microbiome analysis The DNA extraction from the stool samples, the sequencing and analysis of the gut microbiome are explained in this chapter.	7
Biochemical and uremic toxin analysis	
The analysis of the various biochemical values is explained in detail in this chapter as well as summarized in these chapters.	5, 6
The analysis of the various uraemic toxins is explained in these chapters.	6, 7
Anthropometrical analysis	
The analysis of the various anthropometrical measures is explained in these chapters.	5, 6
Statistical analysis	
Each of these chapters explains the various statistical methods used to analyse the data for the different objectives of the study.	5, 6, 7

3.9 Ethics approval

Ethics approval was obtained from the Health Research Ethics Committee of Stellenbosch University (reference number: S18/03/064). Participation was voluntary and participants could withdraw at any point in the study. Participants were enrolled only after they had completed the informed consent form (Addendum A) which was available in English, Afrikaans and isiXhosa. An interpreter was arranged for participants speaking isiXhosa.

Participants were reimbursed for their time and travel. They also received a diet consultation with a qualified dietitian (PI/RA) at their initial assessment and follow-up visits. Names, contact numbers and addresses of participants were kept on a separate sheet stored securely in a locked cabinet. Data was entered into a password-protected Excel spreadsheet; only the RA and PI had access to the spreadsheet for the duration of the study. Data-collection sheets were locked in a filing cabinet after data had been entered into the spreadsheet.

3.10 Institutional approval

Institutional approval was obtained from Tygerberg Hospital management as well as from the nephrology unit head. An online application was made via the National Health Research Database (NHRD) website, which is the national and provincial website for permission to conduct research, which was approved.

3.11 Quality assurance

The RA, a registered dietitian, was trained by the PI to measure weight, height, mid-upper arm circumference, triceps skinfold and waist circumference according to SOPs, examine for oedema, complete the QFFQs and diet-adherence score sheets, collect and check the diet records for accuracy, and complete the questionnaire for socio-demographic and medical information. In addition, the RA was also standardised in advising participants on how incorporate the ß-glucan prebiotic into the diet, how to continue with the diet advised, and how to educate participants to collect their stool samples. Questionnaires were double checked for completion by completing a tick list at the end of the visit and entering the data into the spreadsheet after the visit.

Data entry was done by the RA and double checked by the PI. Descriptive statistics to check for minimum and maximum values was an additional check to see if there were any severe outliers.

The adapted QFFQ and adherence score were sent for content validity to an experienced dietary intake expert dietitian as well as four renal expert dietitians. Changes were made accordingly.

The study was piloted before the study commenced with five participants for face validity to test for the following logistics: use of the questionnaire, explanation to participants on the use of the product and the stool-sampling procedure, dietary

education, blood and stool-sample collection, and delivery to the sites for analysis and storage. The pilot study was also done to determine the time taken for the data collection. These were done at the clinic where the study was to be conducted. The participants were not included in the study sample. No major issues were experienced and minor changes were made to the questionnaire.

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CHAPTER 4: ARTICLE 1 (PUBLISHED)

Keeping the Diet Simple and Natural in Chronic Kidney Disease: A South African-Based Dietary Infographic

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Keeping the Diet Simple and Natural in Chronic Kidney Disease: A South African-Based Dietary Infographic

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Introduction

CHRONIC KIDNEY DISEASE (CKD) is highly prevalent within South Africa.[1] End-stage renal disease requires renal replacement therapies such as dialysis or transplant. However, due to limited resources in the public sector, very few patients are accepted into renal replacement programs.[2] Goals of dietary management aim to treat many of the nutritional complications of the disease including metabolic bone disease, anemia, metabolic acidosis, and malnutrition.[3] It is therefore imperative that measures are sought to delay the progression of the disease as well as minimize and manage symptoms for patients on dialysis. Dietary modification has been shown to assist in this regard.[3] However, adherence to dietary guidelines in all CKD patients is low, and patients often feel that they have nothing left to eat with restrictive lists of foods to avoid.[4,5] The South African population is multicultural, multiethnic, and has a large population from a lower socioeconomic status with many issues regarding food security and dietary diversity.[6] Unemployment and a low level of education affect patients' understanding of their prescribed diet for CKD, limiting their purchasing power and ultimately adherence to their prescribed diet.[7]

Recent studies exploring dietary patterns and its relationship to renal outcomes show some promising results which suggest that CKD diets do not need to be so restrictive.[4,8,9]

Are We Overcomplicating Dietary Advice?

Dietitians in South Africa have established renal exchange lists to calculate individual prescriptions for patients. These exchange lists are comprised of the various food groups with the nutrient breakdown for each group with macronutrient and micronutrient content including phosphate, potassium, and sodium. Although there might be merit to these exchanges, they are very extensive lists to give to educate patients. However, various educational pamphlets have been developed to assist in education purposes which are abbreviated versions of some of the exchanges but not all. There are individual pamphlets for sodium, fluid, potassium, and phosphate. In addition to these pamphlets, meal plans and menus still have to be prescribed for individual requirements, due to meet protein and other nutrient requirements. Due to the extensive amount of information given, patients are often counseled over a few sessions. Other dietary education tools include the Renal Plate and pictorials of foods to avoid and include. Although it is visually displayed, there are still many foods depicted which are not necessary to restrict according to the latest dietary pattern data.

Dietary Patterns in Chronic Kidney Disease

The trials in Table 1 explore the relationship between various dietary patterns and CKD outcomes. Western diets are associated with renal progression, whereas diets that are healthier and rich in fruits, vegetables, and whole grains such as the dietary approach to stop hypertension and Mediterranean diet are associated with a reduction in glomerular filtration rate (GFR) decline. Similarly, trials on patients who have CKD have shown reduced GFR decline and mortality in patients following these diets. Wai et al.[17] suggest that we should not focus on single nutrients alone in CKD diets but rather focus on healthy diets as a whole. With studies showing more favorable effects of healthy diets on CKD outcomes, it is imperative that dietary guidelines be reflective of these findings.

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Table 1. Dietary Patterns and Outcomes in CKD

Study	Patients	Study Design	Outcome
Lin et al. (2011)[10]	3,121 female participants with diet pattern data and urinary albumin-creatinine ratio	Prospective observational cohort study	Western diet patterns associated with significant microalbuminuria and GFR decline. DASH style diet pattern protective against GFR decline
Diaz-Lopez et al. (2012)[11]	665 participants of the PREDIMED trial (high CVD risk)	Cross-sectional	Significant improvement in GFR with Mediterranean diet but no change in the albumin-creatinine ratio (similar benefits to the control low fat diet)
Tyson et al. (2014)[12]	92 participants with stage 1 HPT	Secondary analysis of DASH Na trial	DASH low Na diet showed that neither CKD nor GFR had modified the BP lowering effect of this intervention. Similar effect on BP lowering in those with or without CKD
Huang et al. (2013)[13]	1110 men GFR with CKD	Cohort	Adherence to Mediterranean diet independently predicted survival in CKD
Guitarez et al. (2014)[14]	3,972 participants with CKD	Observation cohort	Southern diets high in take-aways, processed food independently associated with increased mortality, diet rich in fruit and vegetable showed 23% reduced risk of death
Foster et al. (2016)[15]	1,802 participants with CKD	Prospective cohort	Higher diet adherence scores associated with reduced GFR decline
Chen et al. (2016)[16]	14,866 NHANES III participants 5% CKD prevalence	Observational	High protein intake from plant sources was associated with reduced mortality in CKD patients
Wai et al. (2017)[17]	145 patients with CKD stage 3 or 4	Prospective cohort	Healthy diet patterns rich in fruits, vegetables, and limited alcohol consumption are associated with a delay in kidney progression

BP, blood pressure; CKD, chronic kidney disease; GFR, glomerular filtration rate; CVD, cardiovascular disease; HPT, hypertension; DASH, dietary approaches to stop hypertension; NHANES, National Health and Nutrition Examination.

Adherence

The nutritional management in CKD is very complex, considering the intake of protein, energy, and multiple nutrients.[5,18] Healthcare professionals are faced with major challenges to improve patient compliance and adherence to the diet.[18] Findings from studies report that renal diets are unpalatable, very restrictive, and expensive with major lifestyle changes required resulting in frustration, lack of autonomy, and motivation to follow the advice. Patient compliance is poor and they end up relying on convenience and fast foods.[4,5,18] Limitations to the adherence of nutritional prescriptions include weight loss due to low energy intake caused by unpalatable diets, anorexia, difficulty in implementing the diet, and depression.[19] The lack of compliance to dietary restrictions can cause an increase in metabolic waste and fluid in the body resulting in increased morbidity and mortality.[20] Challenges experienced with fluid restriction were due to high temperatures, inability to control their fluid intake, and the patient's wrong perception that dialysis will remove total fluid consumed.[21] The review by Lambert et al.[22] reported that only two-thirds of adults with end-stage kidney disease adhere to recommendations of fluid restrictions and an estimated one in 3 adults are adherent to the renal diet.

Factors known to be associated with good adherence were older age, a higher education level, social and family support, high level of self-efficacy, taste of food, social eating occasions, and dietetic staffing.[22] A randomized control trial by Pisani et al.[18] found that a simplified diet with 6 key dietary messages was better managed by renal patients. An improved metabolic profile of renal disease and the patient's adherence to the diet was seen due to greater flexibility in quantity and quality of food options.

What Is the Evidence Behind Dietary Restrictions?

Protein

According to the latest Italian consensus statements for CKD, unmonitored intake of protein together with other nutrients, exacerbates clinical metabolic alterations related to advanced CKD, as well as reduced efficacy of drug therapy.[19] Recommended guidelines for CKD suggest reducing protein, phosphorus, and sodium; monitoring potassium; and limiting acid load.

There is some controversy in the literature regarding protein restriction in the CKD diet in predialysis patients. However, studies showing no benefits with the reduction in protein are mainly related to non-adherence of these diets.[23] Most nutrition societies worldwide recommend a protein intake of 0.6-0.8 g/kg/day for predialysis renal failure with meta-analysis of large clinical trials showing safety and efficacy.[3] Although these requirements are within normal population protein requirements, albeit at the lower ranges, it must be remembered that healthy individuals have much higher intakes of protein, with the average American intake being up to 1.3 g/kg/day.[24] Therefore, an intake as suggested by most renal guidelines will be lower than the average intake of the population.

There are numerous benefits for protein restriction, including a reduction in waste products, urea, uremic toxins, and acid load. There is also a reduction in oxidative stress, less insulin resistance, reduced phosphate load, an improved lipid profile, and improved metabolic bone disorders.[25] Protein recommendations should prevent malnutrition in patients who are already at a high risk. Fouque et al.[3] suggest that muscle wasting is not necessarily related to reduced protein intake, but rather due to the inflammatory nature of the disease itself, lack of physical activity, as well as an imbalance between protein intake and degradation.[3] Protein intakes slightly lower or close to recommended daily allowance recommendations are acceptable to reduce progression of the disease.

Protein Requirements for Dialysis Patients

Dialysis patients have higher protein requirements compared to predialysis patients. This is mainly due to increased protein losses through the dialysate. Patients that start on dialysis, often struggle to meet the higher protein allowance. Studies have shown dialysis patients to have suboptimal intake of protein, often less than 1 g/day. In peritoneal dialysis the high glucose loads and the constant presence of the dialysate in their peritoneum leaves patients feeling full, which affects their appetite and impacts their ability to meet their protein requirements. Guidelines recommend an intake of up to 1.2 or 1.3 g/kg/day for hemodialysis (HD) and peritoneal dialysis; however, Fouque et al.[3] suggest that recommendations might be too high and that intakes of 1-1.1 g/kg/day are sufficient to maintain normal body composition. A Japanese cross-sectional study in maintenance HD patients did not show improved body composition with intakes greater than 0.9-1.1 g/kg/day. Higher protein intakes are also associated with increased phosphate, potassium, and acid load. Fouque further argues that most of the recommendations upon which the kidney disease quality initiative recommendations were based on, were due to early studies in the 1970s with small sample sizes and variations in the responses to protein intake with some patients doing better on tolerating lower intake of protein. However, recommendations were always based on the higher ranges due to the safety principle and many physicians found that these intakes were not always attainable. Therefore, slightly lower protein requirements might be more practical and acceptable, with no significant effect expected on body composition (Table 2).

Phosphate

Hyperphosphatemia in patients with renal failure can be the result of several conditions: a low dialysis dose, uncontrolled hyperparathyroidism paired with dialysis vintage, or a low parathyroid hormone level (usually post parathyroidectomy). The imbalances in the calcium-phosphate-parathyroid hormone axis cause rapid vascular aging, decreased bone mineral density, left ventricular hypertrophy, and increased risk of death.[9] CKD-bone mineral disease is associated with decreased physical function, increased risk of fractures, and reduced quality of life.[4]

Overview of Current Guidelines

The current clinical management of hyperphosphatemia is focused on dialysis treatment, phosphate binder therapy, and dietary phosphate restrictions. Dietary phosphate restriction presents a cornerstone of the renal diet and the treatment of CKD-bone mineral disease.[4,9,26-30]

Dietary phosphates can be divided into 2 types: organic phosphate is found mostly as phosphoproteins and membrane phospholipids in animal sources (e.g., meat and dairy) and as phytate in plant-based sources (e.g., legumes, whole grains, and nuts). Organic phosphate is hydrolyzed in the intestinal tract and then absorbed into circulation as inorganic phosphate.

Animal sources including meat, poultry, fish, eggs, and dairy products have an absorption rate of 40%-80% (being higher when vitamin D is present). In the non-vegetarian western diet, more than half of the dietary phosphate load originates from animal protein.[30,31]

Plant-based sources including beans, peas, cereal, and nuts have an absorption rate of 20%-40%. Most of the phosphate is found as part of the phytate, which must be hydrolyzed by phytase to be released and absorbed in the small intestine. Phytase however is not expressed in the small

Table 2. Protein Recommendations in CKD

Recommended Range[3]	Observations
Protein predialysis 0.8 g/kg suggested	Protein restriction is suggested to slow progression of disease
Protein in dialysis 1 g/kg minimum, preferably 1.2 g/kg	Although many recommendations advise higher protein amounts, many patients do not practically achieve to this goal

intestine; therefore absorption of phytate containing foods is even more limited.[30]

Inorganic phosphates is used as an additive or preservative in processed foods to increase palatability and shelf life. Inorganic phosphates have an absorption rate of more than 90%. Phosphate is the main component of several additives (phosphoric acid, phosphates, and polyphosphates) and used in industrial food processing to extend conservation, enhance color or flavor, and retain moisture. The food industry is not required to report quantities used in their products, merely to list the additive in the ingredient list. Current regulations allow reporting the presence of phosphate containing additives, preservatives, thickeners, and emulsifiers used, as an abbreviation (as the ''E'' series) or by their full name. This generally leads to an underestimation of dietary phosphate intake.[9] The intake of phosphate coming from processed foods may reach as much as 1000 mg/day.[30]

Phosphate concentrations in both animal and vegetable sources also may be reduced with some cooking methods, such as boiling, slicing, and pressure-cooking.[4] Boiling of food reduces phosphorus by 51% in vegetables, 48% for legumes, and 38% for meat.[5,19,32]

Fiber

In advanced CKD, a state of dysbiosis of the intestinal microbiota occurs due to altered intestinal permeability and an imbalance of microbial metabolism with an increased production of uremic toxins like *p*-cresol and indoxyl sulfate. Dietary restriction of plant-based proteins and fiber worsens dysbiosis. The uremic toxins, depending on the stage of CKD, accumulate in the patient and contribute to an accelerated progression toward renal death, inflammation, and cardiovascular (CVD) complications.[19] Markers of inflammation and all-cause mortality have been shown to be reduced by a high dietary fiber intake which is related to its antioxidant anti-inflammatory properties and altered bacterial metabolism in the colon.[8] It is therefore recommended for patients with advanced CKD to restrict protein intake and obtain a fiber intake of 20-30 g/day. The caring for Australians with renal impairment (CARI) guideline 2013 recommends that patients with early CKD consume a diet rich in dietary fiber (Grade 2D evidence).[33] Insoluble fiber has been shown to improve biochemical markers like urea and creatinine in HD patients. Therefore, increasing intake of insoluble fiber can be especially beneficial in these patients as their intake has been shown to be very low.[34]

Sodium

Sodium is the main cation in the extracellular space and a key contributor to plasma osmolarity. Excessive dietary sodium intake or treatment-related factors (usage of high-sodium dialysate solutions or hypertonic saline infusions used to prevent cramping and intradialytic hypotension) may contribute to changes in plasma osmolarity.[4]

Sodium plays a vital role in regulating blood pressure and blood volume through the renin-angiotensin-aldosterone system, which is impaired in patients with CKD who are also salt sensitive and may lead to hypertension (HTN).

High dietary sodium intake increases proteinuria, reduces the effectiveness of antiproteinuric therapies (especially renin-angiotensin-aldosterone system inhibitors), attenuates the antihypertensive effects of most antihypertensive therapies, and increases medication use, especially diuretics.[5,19,23] Hypernatremia also results in an increase in thirst, which in turn is associated with higher interdialytic weight gain, predialysis systolic blood pressure, and chronic fluid overload. These factors are associated with acute and chronic CVD complications, progression of CKD, and mortality.[4]

Overview of Current Guidelines

It is well recognized that HTN and related CVD comorbidities can be controlled in many HD patients through nonpharmacological means. Regulating sodium intake is essential for a population in which HTN is almost always present.[19]

Sodium in the form of sodium chloride (salt) is found naturally in food and is often used in large quantities to process and preserve food.[23] Dietary sodium restriction in conjunction with persistent ultrafiltration can limit extracellular volume expansion and HTN in HD patients without the need of antihypertensive medications. Protocols as described in Biruete et al.[4] to control HTN combine the use of dialysis sessions (ranging from 4 hours up to 8 hours) with sodium-restricted diets. These protocols report up to 90% of patients controlling BP without the use of antihypertensive medications and lower mortality rates. Sodium restriction may be beneficial as long as nutritional status and food intake are not compromised. Current advice is based on the cornerstone that the CVD benefits of sodium restrictions outweigh the potential concerns (Table 3).[4,35]

Fluid

In CKD the cornerstone to volume management is the removal and control of excess fluid.[21] Fluid intake is mainly driven by thirst due to dietary salt intake and other factors like high blood glucose in patients with diabetes, angiotensin II, potassium depletion, and psychological factors. In CKD the combined intake of salt and water results in impaired volume homeostasis and is usually associated with HTN, proteinuria, and progression of renal disease.[36-39] However, patients on HD that are anuric will find it more challenging to manage their thirst.[22]

Table 3. Sodium Recommendations in CKD

Recommended Range[5,23,27,33]	Observations
<2.5 g/d (109 mmol/d)	Less fluid intake may be difficult to adhere to if patients are to eat larger portions of protein and kcal
<2.3 g/d (<100 mmol/d)	A no-added-salt diet in conjunction with antihypertensive medication may appear to be safe, feasible, and cost-effective for reducing CVD risk, CKD progression, and dosage of antihypertensive medication
Sodium predialysis <100 mmol/d	Early CKD patients should restrict their dietary intake to reduce blood pressure and albuminuria
Sodium dialysis < 2.4 g/d or < 100 mmol/d	Restricting dietary sodium intake should be recommended in most adults with CKD and HTN

CKD, chronic kidney disease; CVD, cardiovascular; HTN, hypertension.

Overview of Current Guidelines

It is recommended that patients drink fluids in moderation (Table 4).[39] Salt restriction should also be advised to patients who consistently present with a high interdialytic weight gain, after excluding postdialysis volume depletion or uncontrolled blood glucose, and allow increased time on dialysis to achieve normal volume status.[38]

Potassium

Hyperkalemia seen in CKD patients is known to be a direct and life-threatening complication caused by intrinsic factors, e.g., reduced GFR, impaired mineralocorticoid activity and metabolic disturbances, like acidemia or hyperglycemia, and extrinsic factors, e.g., drugs like renin-angiotensin-aldosterone system inhibitors and dietary potassium intake.[40] In cases where hyperkalemia remains to be problematic, it has been associated with acidosis, gastrointestinal bleeding, uncontrolled diabetes mellitus, or iatrogenic effects.[9] In HD patients a weak correlation was found between potassium intake and serum potassium concentrations. Only about 2% of the variance in quarterly mean predialysis serum potassium concentration can be explained by reported dietary potassium intake.[30] In the Balance_Wise study, there was also no significant correlation found between serum potassium concentration and potassium intake and remained nonsignificant after adjusting for age, sex, race, and body mass.[41]

Table 4. Fluid Recommendations in CKD

Recommended Range[5,32,39]	Observations
Early CKD/predialysis 2.0-2.5 L/d	Including fluid from food
500 mL plus urine output	Drink fluids in moderation
HD ≥ 1 L urine (2.0 L/d)	Caution should be taken against overzealous salt and fluid restrictions in dialysis patients with significant residual renal function and urine output present
HD ≤ 1 L urine (1-1.5 L/d)	
HD anuric 1 L/d	
PD 1-3 L/d	

CKD, chronic kidney disease; HD, hemodialysis; PD, postdialysis.

Overview of Current Guidelines

In CKD restricting dietary potassium intake is not fully supported in literature due to its known CVD benefit, while the intake of plant-based food is more advised (Table 5).[9] Very little is known about the hidden food sources and quantity of potassium and phosphate, known to be high in processed and preserved foods, including other preservatives, taste enhancers, and colorants. Therefore, the nutritional content of processed foods is considerably different from natural whole foods. A salt substitute of 5-6 g can supply additional potassium from 1,000-1,200 mg to 1,500-1,800 mg, therefore its intake should be limited.[19,40]

Liberalizing the Diet

Diets rich in plant-based protein have been shown to provide additional benefits to CVD health, blood pressure, cholesterol metabolism, decreased production of uremic toxins, and the progression of CKD.[16,30] Plant-based food that is higher in fiber including whole grains, legumes, fruits, vegetables, and nuts should be advised.[9,34,44] On the other hand, processed, preserved, and ready-made or convenience foods should be limited due to additives and preservatives high in potassium and phosphate or salt substitutes used.[19,29,43]

Patient's should be educated on reading labels to identify additives listed and using appropriate cooking techniques like soaking and boiling to help reduce potassium and phosphate content in food.[19] A practical approach would be to shift the focus of nutrition counseling toward educating and

Table 5. Potassium Recommendations in CKD

Recommended Range[5,9,19,33,42,43]	Observations
CKD predialysis (stage 1-5) <3-4.7 g/d	Potassium is not restricted unless levels are increased. Most K-rich foods are heart-healthy. Advise intake of plant-based food
HD 2.7-3.1 g/d	Adjustments to intake are based on serum levels
PD 3-4 g/d	Adjustments to intake are based on serum levels

CKD, chronic kidney disease; HD, hemodialysis; NKF, National Kidney Foundation; PD, postdialysis.

empowering patients to make informed choices regarding natural and whole foods that are within the ''heart-healthy'' range.[5]

Development of an Infographic for Patients With CKD

Based on the research, a simplified infographic was developed for educational purposes for CKD patients. The following steps were followed:

- Research literature for the latest CKD patient guidelines and include articles about diet liberalization to improve compliance and general health benefits.
- Discuss with expert CKD nutrition panel the main dietary or nutritional messages from the research.
- Decide and formulate short simplified messages from the research to include in infographic.
- Compile a draft infographic in text and select appropriate images.
- Have infographic designed professionally by a graphic designer.
- Review by expert CKD nutrition panel and recommend changes where necessary.

Conclusion

Research has shown that diets that are more liberal in fruits, vegetables, and whole grains have benefits that outweigh the risks in CKD patients. A South African based infographic aimed to simplify the diet and adapted specifically for CKD has been developed which might assist in improving compliance to the diet and ultimately improve CKD outcomes.

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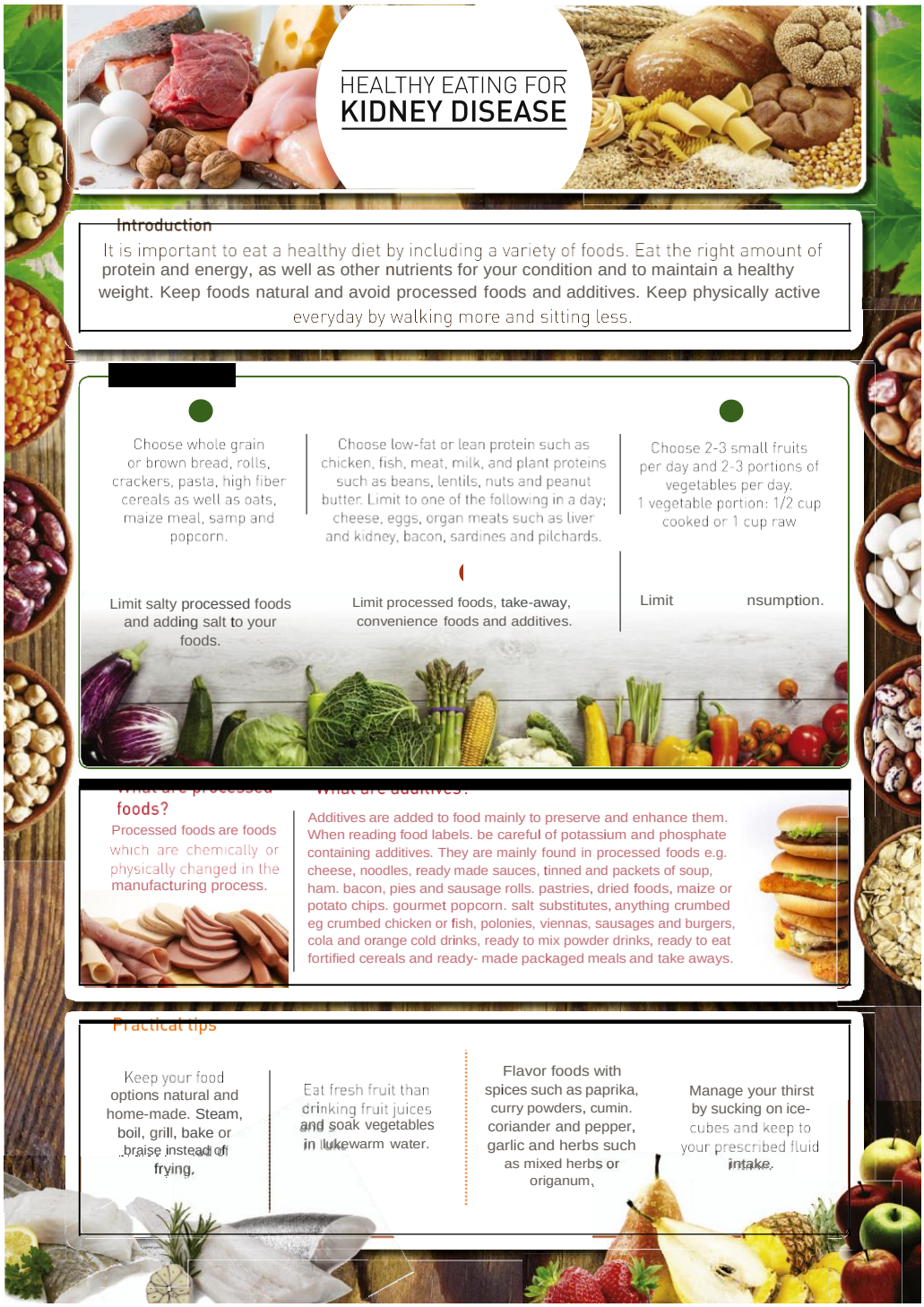

CHAPTER 5: ARTICLE 2 (PUBLISHED)

Obesity and Other Nutrition Related Abnormalities in Pre-Dialysis Chronic Kidney Disease (CKD) Participants

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Obesity and Other Nutrition Related Abnormalities in Pre-Dialysis Chronic Kidney Disease (CKD) Participants

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Abstract: Chronic kidney disease (CKD) is increasing in sub-Saharan Africa. Undernutritionhas been prevalent amongst end stage CKD patients, with limited data on the prevalence of obesity. The aim of this study was to assess the nutritional status of CKD patients using various methods sensitive to over and under-nutrition. Stage 3 to 5 CKD patients (glomerular filtration rate (GFR) < 60 mL/min/1.73 m^2) attending a pre-dialysis clinic in Cape Town, were enrolled. Exclusion criteria included infectious and autoimmune conditions. Sociodemographic, clinical and biochemical data were collected, and anthropometric measurements were performed. Dietary intake was measured with a quantified food frequency questionnaire (FFQ). Statistical Package for the Social Sciences (SPSS) version 26 was used for statistical analysis. Seventy participants, with mean age of 41.8 ± 11.8 years,52.9% females and 47.1% males were enrolled. Participants enrolled mainly had stage 5 kidney failure. Thirty percent were overweight (21) and 25 (36%) were obese, 22 (60%) of females were overweight and obese, while 13 (39.4%) of males were predominantly normal weight. Abdominal obesity was found in 42 (60%) of participants, mainly in females. Undernutrition prevalence was lowat 3%. Dietary assessment showed a high sugar and protein intake. There was a high prevalence of overweight, obesity and abdominal obesity in CKD stage 35 patients, with unhealthy dietary intakeand other nutritional abnormalities.

Keywords: pre-dialysis nutrition assessment; nutrition assessment in chronic kidney disease;obesity in chronic kidney disease

1. Introduction

Malnutrition in the form of overweight and obesity has been increasing in prevalence in chronic kidney disease (CKD) [1,2]. This is likely due to the increase in chronic diseases of lifestyle, since many are risk factors for the development of CKD [2,3]. Obesity is closely related to cardiovascular and other metabolic diseases such as insulin resistance, type 2 diabetes mellitus and chronic inflammation through various mechanisms [3,4]. Obesity also induces glomerular hyperfiltration [3]. An increased body mass index (BMI) above 25 kg/m^2 has recently been associated with a progressively increased risk of CKD stages 4 to 5 [5]. It is therefore important to identify and treat obesity in early CKD to prevent further deterioration of the disease.

CKD results in many nutritional status abnormalities as glomerular filtration rate decreases. These include bone mineral abnormalities, anemia, inflammation, electrolyte imbalances, undernutrition, increased catabolism and appetite problems [6,7]. This results in many dietary restrictions. Due to the restrictive nature of the diet and due to symptoms of the disease, diet adherence is low [8]. These factors in addition to obesity will negatively influence the nutritional status of CKD patients.

The International Society of Renal Nutrition and Metabolism (ISRNM) developed a set of criteria focusing on body weight, muscle mass, biochemical assessment and dietary intake to diagnose undernutrition, specifically in CKD patients. The Subjective Global (SGA) assessment tool has also been used to diagnose undernutrition in CKD; however due to its subjectivity, the ISRNM suggest that it not be used to diagnose undernutrition, but should rather be used as a clinical marker [9,10]. Neither of these have criteria for identifying overweight or obesity as a malnutrition risk.

The main focus of research has been on the high prevalence of malnutrition in terms of protein energy undernutrition in pre-dialysis CKD patients [6,11,12]. The ISRNM criteria are used to diagnose undernutrition in some of the studies [9]. The KoreaN cohort study for Outcome in patients With Chronic Kidney Disease (KNOW-CKD) study reported a prevalence of 9%, while a Nigerian study reported a prevalence of undernutrition of 47% using the ISRNM criteria, however the two studies used the criteria in different ways [13,14]. No information was provided for the rest of the BMI categories for either studies, although the average BMI approached the overweight category. Studies using the SGA has also found high rates of undernutrition [1,10,15]. These studies demonstrate that different screening criteria are used to diagnose undernutrition prevalence and that there is a failure to report on the extent of overweight or obesity.

It is possible that less emphasis is placed on obesity because of the obesity paradox; this refers to the reduced mortality outcomes that has been shown in CKD with higher BMI's [16]; however there are conflicting results particularly within the pre-dialysis patient group. Herrington et al. [5] suggests reasons why the obesity paradox theory may not be substantiated in CKD; these include a low BMI due to individuals being in an overall poorer health condition and often in a cachectic state, they also suggest methodological and enrolment flaws in some of the studies reporting these associations.

This study assessed the nutritional status of CKD pre-dialysis participants using different measures that may be sensitive to both over and undernutrition.

2. Materials and Methods

This cross-sectional study investigated the nutritional status of 70 stage 3 to 5 CKD participants attending the pre-dialysis clinic at Tygerberg Hospital in Cape Town, South Africa, between 1 August 2018 and 30 September 2019. Participants files were screened and were selected if their GFR's were less than 60 mL/min/1.73 m^2 at their routine Renal Clinic visit and they were older than 18 years, provided exclusion criteria did not apply. The latter included any infectious diseases that may affect nutritional status, immunological conditions, severe gastrointestinal disease, pregnancy, diabetes and participants who were expected to start dialysis in the next 2 months. This study represents the baseline data of a randomized control study investigating uraemic toxins, gut microbiome and CKDoutcomes using a prebiotic supplement. A total of 70 participants were enrolled for the study and the baseline data is presented here.

Ethics approval was obtained (S18/03/064) from Stellenbosch University Health Research Ethics Committee.

Data collected included socio-demographic and relevant biochemical and clinical information.

The following anthropometric measurements were performed according to World Health Organization (WHO) standards [17]: weight (kg), height (cm), waist circumference (cm), mid-upper arm circumference (MUAC) (cm) and triceps (mm) [18] and reported by group and gender. Appendix A indicates the formulae used to calculate the values, reference values and interpretation.

Dietary data were collected by using a 160 item Food Frequency Questionnaire (FFQ) to evaluate the patient's dietary intake. It was adapted from a FFQ used in a South African study to include a greater variety of fruits, vegetables and phosphate containing foods and was evaluated for face and content validity [19]. It was administered by the dietitian researcher who interviewed participants. Food models and household metric measuring instruments such as spoons and cups were used to estimate portion sizes. Codes of the food items and the portion sizes were captured as daily intake. It was analyzed for the macronutrient and micronutrient composition per person per day,

the SAFOODS database was used to analyze the data [20]. The mean/median dietary intake of nutrients were assessed and compared to the Kidney Disease Outcomes Quality Initiative (KDOQI) and Kidney Disease Improving Global Outcomes (KDIGO) recommendations [21–23].

Adjusted oedema free body weights were used to calculate the required nutrients where values were given per/kg of weight for energy and protein and as a percentage of energy for carbohydrate and fats, calculated in grams per day. Values or ranges for a specific nutrient were given as per the guidelines for cholesterol, fiber, vitamins and minerals. The nutrients were expressed as nutrient intake below, above and within recommendations as a percentage of participants.

Patient's barriers to eating healthy were assessed with the responses being binary: the cost of food, time to cook or shop, motivation to cook or shop, lack of family support and the availability of shops to purchase food.

Data were tested for normality using various methods including Kolmogorov-Smirnoff, skewness and kurtosis values, histograms and Q-plots.

Basic descriptive tests were performed using frequencies and percentages for categorical data, means and standard deviations for normally distributed continuous data and medians and interquartile ranges for continuous data that was not distributed normally.

The following analytical tests were employed: correlations between protein intake and urea and creatinine, and between energy and urea and creatinine, as well as gender differences for anthropometry using t-tests and Chi-squared tests. Analysis of variance compared the following: BMI categories and dietary intake, financial income and dietary intake and between GFR and age, BMI, protein and energy intake. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Socio-Demographics

Seventy participants entered the study. The mean age of the participants was 41.7 ± 11.8 years, with a slight predominance of females (53%). Most participants were employed, earning less than US $126 and had up to grade 11 schooling (Table 1).

3.2. Clinical

Hypertension was the most prevalent cause of renal failure and occurred in 35 (50%) of participants. Most participants had no oedema (44, 62.9%) and 31 (44.2%) had stage 5 CKD. The mean systolic blood pressure was 146.0 ± 25.5 mmHg and diastolic pressure was 81.0 ± 15.3 mmHg. (Table 2).

Fifty-one (73.9%) participants were receiving diuretics, 42 (60.9%) ACE inhibitors and 38 (55.1%) calcium channel blockers. Nearly a third of participants were on calcium and iron supplements. The majority of participants were on multiple medication combinations.

3.3. Anthropometry

Table 3 shows the mean weights which were significantly different (p = 0.03) for males and females. Similarly, the mean triceps were higher in females than males (p = 0.001).

The mean BMI was in the overweight category for the group with no gender differences. Regarding the BMI categories, 21 (30%) participants were overweight and 25 (36%) were obese. Obesity was more prevalent in females, with 46% of females being obese; whereas males were mainly in the normal weight category, with differences in gender being statistically significant (Chi^2 = 8.9, p = 0.03) (Table 3).The mean MUAC for the group was in the obese category, with no gender differences.

The prevalence of underweight in this study was 4.3%, with only 3% of participants also having a wasted muscle and fat mass.

The majority of participants had a waist circumference in the high risk for chronic diseases category; this group comprised predominantly of females (57%), whereas males were in the normal waist category. The gender differences in waist circumference was significant (Chi^2 = 8.0, p = 0.005).

Thirty six (51.4%) of participants, had average arm muscle evenly spread between gender, and 40 (57.1%) also had average arm fat, with a significant gender differences between AFA categories (Chi^2 = 12.2.0, p = 0.02).

Table 1. Sociodemographic profile of participants.

		n	**Mean ± SD**
Age (years)		70	41.7 ± 11.8
		n	Percent %
Gender	Male	33	47.1
	Female	37	52.9
Employment status	Full time	29	41.4
	Part time	5	7.1
	Unemployed	22	31.4
	Pensioner/Grant holder	4	5.7
	Other	10	14.2
Monthly Income	US $0–126	29	41.4
	US $127–316	18	25.7
	US $317–633	15	21.4
	US $634–949	5	7.1
	>US $949	3	4.3
Education level	Primary school	10	14.3
	Grade 8–11	32	45.7
	Grade 12	20	28.6
	University	1	1.4
	Technicon	7	10.0

Table 2. Clinical data of participants.

		n	**Mean ± SD**
Blood pressure (systolic) mmHg		64	146.0 ± 25.5
Blood pressure (diastolic) mmHg		64	81.0 ± 15.3
		n	%
Oedema	None	44	62.9
	Mild	15	21.4
	Moderate	8	11.4
	Severe	3	4.3
GFR stages	Stage 3	21	30.0
	Stage 4	18	25.7
	Stage 5	31	44.2
Cause Renal Failure	Polycystic kidney disease	6	8.6
	Hypertension	35	50.0
	Glomerular disease	13	18.6
	Other and unknown	16	22.9

GFR: Glomerular filtration rate.

Table 3. Anthropometry for total group and gender.

	Total Group n = **70**	**Male** n = **33**	**Female** n = **37**	* p **Value**
			Mean ± SD	
Weight (kg)	76.8 ± 25.4	82.9 ± 23	71.4 ± 19.7	* 0.03
BMI (unit)	28.4 ± 7.0	28.4 ± 7.8	28.6 ± 6.4	* 0.90
Waist circumference (cm)	92.1 ± 16.8	94.9 ±19.5	91.6 ± 13.7	* 0.18
MUAC (cm)	31.0 ± 5.4	31.2 ± 5.1	30.5 ± 5.8	* 0.84
Triceps (mm)	21.0 ± 9.1	17.0 ± 9.0	24.0 ± 8.0	* 0.001
BMI Categories			n (%)	
Underweight	3 (4.3)	0	3 (8.1)	
Normal weight	21 (30.0)	13 (39.4)	8 (21.7)	Chi^2 = 8.9, p = ** 0.03
Overweight	21 (30.0)	12 (36.4)	9 (24.3)	
Obese	25 (35.7)	8 (24.2)	17 (45.9)	
Waist circumference Categories				
Normal	28 (40)	18 (54.5)	10 (27.0)	
Increased risk	13 (18.6)	7 (21.2)	6 (16.2)	Chi^2 = 8.0, p = ** 0.005
High risk	29 (41.4)	8 (24.2)	21 (56.8)	
MUAC Categories			n (%)	
Undernourished	5 (7.1)	0	5 (13.5)	
Normal	17 (24.3)	9 (27.2)	8 (21.6)	Chi^2 = 3.0, p = ** 0.22
Overweight	9 (13.0)	5 (15.1)	4 (10.8)	
Obese	39 (55.7)	19 (57.5)	20 (54.0)	
AMA Categories			n (%)	
Wasted	1 (1.4)	1 (3.0)	0	
Below average muscle	7 (10.0)	6 (18.2)	1 (2.7)	
Average muscle	36 (51.4)	17 (51.5)	19 (51.4)	Chi^2 = 8.9, p = ** 0.06
Above average muscle	13 (18.6)	5 (15.2)	8 (21.6)	
High muscle	12 (17.1)	3 (9.1)	9 (24.3)	
AFA Categories			n (%)	
Wasted	5 (7.1)	3 (9.1)	2 (5.4)	
Below average fat	5 (7.1)	1 (3.0)	4 (10.8)	
Average fat	40 (57.1)	18 (54.5)	22 (59.5)	Chi^2 = 12.2, p = ** 0.02
Above average fat	10 (14.3)	2 (6.1)	8 (21.6)	
Excess fat	9 (12.9)	8 (24.2)	1 (2.7)	

* Independent *t*-tests. ** Chi-squared tests. BMI: body mass index; MUAC: mid upper arm circumference; AMA: arm muscle area; AFA: arm fat area.

3.4. Dietary Intake

Table 4 describes the mean/median intake of macronutrients and micronutrients and how they compare to recommended KDOQI ranges. The mean energy intake was 27 kcal per kg which is within the guidelines. The mean protein intake was higher than the guidelines at 1 g/kg, specifically animal protein at 64.8% of protein intake. Saturated fat intake was higher than guidelines at 10.7%.

Added sugar and total sugar intake was high at 39 g and 70 g respectively. The mean intake of all other minerals and vitamins were within the recommendations, except for folate and vitamin D, which were lower than the guidelines.

Table 4. Recommended intakes versus actual intake of nutrients.

	Recommended Daily Allowances [21]	**Actual Intake Mean ± SD** ***n* = 70**
Energy kcal/kg	25–35 [23]	27 2041.7 ± 732 kcal/kg
Total protein g/kg	0.6–0.8 0.55–0.6 g/kg [23]	1 74.2 ± 28.4 g
Plant protein	50% of protein intake	34.2% 25.4 ± 10.7 g
Animal protein	50% of protein intake	64.8% 48.1 ± 21.2 g
Total fat	34% Energy	35.2% 80.0 ± 34.9 g
Saturated Fat	<7% of Energy	10.7% 24.3 ± 11.7 g
Monounsaturated Fat	<20% Energy	12.2% 27.7 ± 14.4 g
Polyunsaturated Fat	<10% Energy	9.0% 20.6 ± 8.6 g
Total trans fat g	0	0.7 ± 0.5
Cholesterol mg	200–300	278.2 ± 133.7
Carbohydrate	55% Energy	49.3% E 251.9 ± 93.7 g
Added sugar g	25	39.1 (23.0, 59.1) *
Total sugars g	NA	69.9 ± 29.2
Total dietary fiber g	253–0	21.8 ± 9.7
Calcium mg	1000–1200 800–1000 [23]	484.7 (349.0, 743.1) *
Iron mg	101–8	13.0 ± 4.6
Phosphate mg	800–1000	1038.7 ± 420.6
Sodium mg	2400 2300 [23]	2049 ± 965.1
Potassium mg	2000–3000	2691.2 ± 932.7
Vitamin B6 mg	5	3.2 ± 1.3
Folate mg	1000	291.8 ± 118.0
Vitamin D mg	5–10	2.7 (1.8, 5.2) *

All values given as a mean SD, except as * median (interquartile range). Values calculated where g/kg of a nutrient is given or as range. Updated KDOQI guidelines were recently released: the reference [23] indicates where they differ, for the rest of the values, no specific values were provided in the update, it is therefore based on previous guidelines. NA, not applicable.

Barriers such as cost of food were prevalent in 63% of patients, and 25% of patients lacked motivation to shop or cook.

We compared the intake of nutrients to dietary recommendations and grouped them according to the following criteria: within, more than or less than the recommended guidelines. (Figure 1). The following nutrients were consumed in quantities higher than the recommendations in more than50% of participants: saturated fat, added sugar, trans fats, animal protein and total protein.

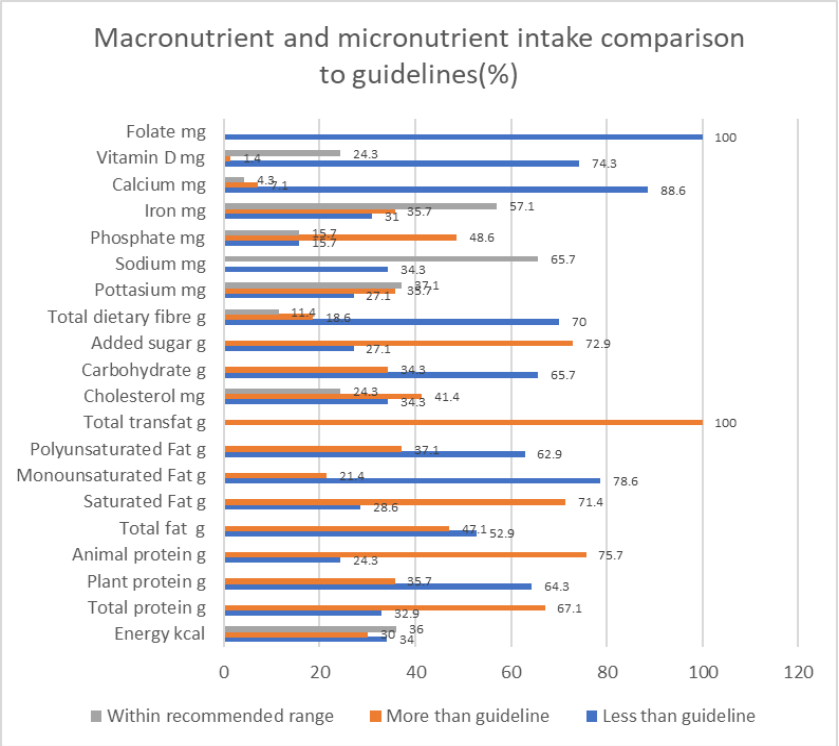

Figure 1. Nutrient intake compared to guidelines (% of participants).

The following nutrients were consumed less than the recommendations in more than 50% of participants: vitamin D, calcium, fiber, folate, carbohydrates, total fat, polyunsaturated, monounsaturated fat and plant protein. Interestingly, of the 34% of participants with energy intakes less than recommendations, 16% were in the obese BMI category, with an average energy intake of only 14.3 ± 2.93 kcal/kg.

A significant association was found between socioeconomic status and protein intake ($p = 0.03$), with the highest protein intake being in the second highest income (US $634–949) category (Figure 2). No other significant associations were found between socioeconomic status or BMI categories and any other macronutrient intakes.

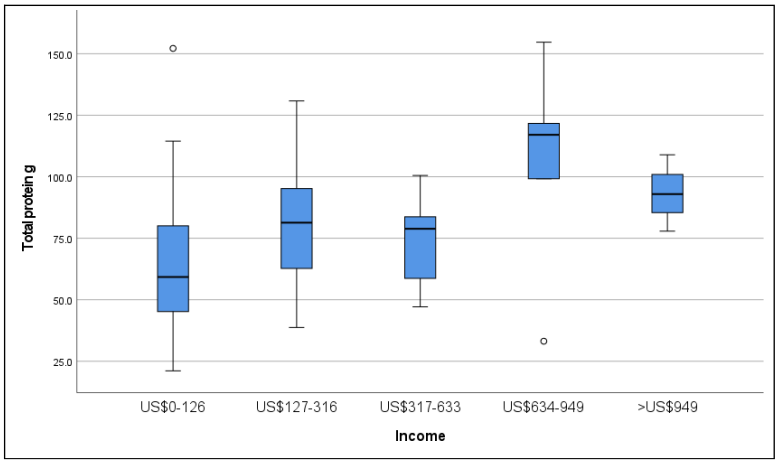

Figure 2. Boxplot of protein intake and socioeconomic status; ○ Indicates outliers.

3.5. Biochemistry

The median urea and creatinine were raised and the GFR was low, which is consistent with end stage CKD. The median potassium, sodium and phosphate were in the normal ranges. The total cholesterol and low-density lipoprotein (LDL) were raised according to high risk cut off criteria. The median CRP was higher than the cut-off range and inflammation was present in 42 (60%) of participants. (Table 5) GFR categories (stage 3, 4 and 5) were compared to various variables, including age, BMI, waist, MUAC, protein and energy intake, with no significant differences found. Even though the BMI of those with stage 4 and 5 kidney failure were higher than those in Stage 3 kidney failure, the difference was not significant ($p = 0.3$). Similarly, waist circumference was also 8 cm higher in stage 4 than stage 3, also not statistically significant ($p = 0.2$). As expected, biochemical values were significantly higher for urea ($p < 0.01$), creatinine ($p < 0.01$), phosphate ($p < 0.01$) and potassium ($p = 0.03$) in stage 5 compared to stage 3 kidney failure due to the more advanced stage of the disease.

Table 5. Biochemistry profile of participants.

	Normal Ranges *	Actual Median and Interquartile Range
Urea mmol/L	2.1–7.1	16.3 (10.9, 25.3)
Creatinine umol/L	64–104	287.0 (183, 477.5)
GFR mL/min·1.73 m²	>60	19.0 (10.8, 31.2)
Potassium mmol/L	3.5–5.1	4.8 (4.3, 5.2)
Sodium mmol/L	136–141	142.0 (139, 144.0)
Phosphate mmol/L	0.78–1.42	1.4 (1.1, 1.5)
Total Chol mmol/L (high risk)	<4.5	4.9 (3.9, 5.7)
LDL (high risk) mmol/L	<2.6 **	2.7 (2.1, 3.3)
HDL mmol/L	>1.2	1.1 (1.0, 1.4)
TG mmol/L	<1.7	1.7 (1.2, 2.5)
CRP mg/L	<3 **	5.0 (1, 9)

* Normal ranges used by the South African National Health Laboratory (NHLS). ** Inflammation defined as a CRP > 3 mg/dL [13]. ** According to the American College of Cardiology CKD stage 3 and 4 is considered high risk for artherosclerotic cardiovascular disease [24]; GFR: glomerular filtration rate; Total Chol: Total cholesterol; LDL:Low-density lipoprotein, HDL: High density lipoprotein, TG: Triglycerides; CRP: C-reactive protein.

4. Discussion

The aim of this study was to assess the nutritional status of pre-dialysis CKD patients. We found a high prevalence overweight, obesity and abdominal obesity, low rates of undernutrition, and an unhealthy diet.

The mean age of the participants were 41.7 years, which is younger than most other CKD studies. In contrast to older studies [14,15], almost two thirds of participants were overweight or obese; 45% of females were predominantly obese. Abdominal obesity was present in 60% of participants, again predominantly in females. The BMI and waist circumference were higher in stage 4 and 5 CKD. Chan et al. [1], similarly to our study showed 62.4% of participants were overweight and obese, whereas Dierkes et al. [2] found an overweight and obesity prevalence of 65%. They also found a high rate of central obesity in 53% of patients. Epidemiological studies have also shown high prevalence of CKD in overweight and obese patients with higher BMIs in CKD stages 4–5 [5,16]. The high prevalence of overweight and obesity could relate to the high prevalence in the general population in South Africa in which 68% of women and 31% of men are overweight or obese [25]. The prevalence found in this study is similar to that of the background South African population in women, but there is a much higher prevalence of overweight and obesity in CKD men in this study. Most of these patients may have already been overweight and obese at diagnosis of CKD. This emphasizes the importance of weight control during the early stages of kidney disease to prevent further deterioration of the disease [3].

A majority of participants in the study had high arm muscle and high fat area which could relate to the increased BMI as other studies have shown [26,27]. Triceps and MUAC measurements were higher than reported [1,15,28] Muscle wasting was not found in obese participants in this study. Chan et al. [1], found a high rate of malnutrition including muscle wasting in their overweight and obese participants based on the SGA. The malnutrition was more significant as the age increased [1]. Dierkes et al. [2] also found a high rate of sarcopenia and sarcopenic obesity in their study, which also increased with age. Body composition changes associated with aging include increased fat mass and reduced muscle mass [2].

A possible explanation for the lack of muscle wasting and sarcopenia is this study is that the participants were much younger with an average age of 41 than the participants in both of the other studies, who averaged 65 years of age.

Hypertension was the most prevalent cause of CKD in participants as documented by the clinicians; this was similar to other studies and is also reflective of the hypertension rates in South Africa, where hypertension is present in 45% of men and 44% of women [13,14,25]. Overweight and obesity can account for 65–75% of the risk for hypertension [3]. Renal sinus fat have been associated with hypertension and the need for more hypertensive medications [3] The blood pressure was slightly higher in this study than recommended in CKD participants despite being on various combinations of anti-hypertensive medications. Hypertension together with other disorders linked to metabolic syndrome such as the obesity shown in this study can act synergistically to increase the risk for CKD and end stage kidney failure (ESKF). Sodium intake was lower than recommendations in a majority of participants. Sodium has been linked to hypertension and fluid retention.

Undernutrition in CKD has been a serious feature of CKD participants whereas obesity has received less attention due to the obesity paradox concept. Recent studies still show a high prevalence of undernutrition in some populations [1,14,15]. However, these studies use the criteria differently, leading to varying results. When the criteria used in the ISRNM were applied in our study, i.e., total cholesterol <100 mg, BMI <23 kg/m^2 and dietary protein intake <0.6 g/kg, the prevalence of undernutrition was found to be zero. However, there were participants that were underweight as well as having wasted arm muscle mass and fat. The overall rate of undernutrition was therefore very low in this study and mainly confined to underweight participants with a wasted muscle and fat arm area which affected only 3% of participants. This is similar to that reported by Dierkes et al [2] who only found malnutrition in 3% using a nutritional risk score.

The dietary intake was indicative of unhealthy food choices, which is typical of a Western dietary food pattern. This study differs from others in terms of energy intake: 30% were higher than recommended range, while 32% were lower and 34% were in the normal range. Wlodarek et al. [29] reported that as many as 93% of their participants consume less energy than recommended. Steiber et al. [30] showed only 15% of participants met 75% of their requirements. Energy intake did not differ significantly amongst the BMI or GFR categories. A quarter of obese participants in this study were taking an average energy intake of 14 kcal/kg which may reflect underreporting. The latteris a common finding in obese patients [31]. The KDOQI energy requirement for obese participants are 30–35 kcal/kg [21]. More recent guidelines have advised 25–35 kcal/kg based on age, sex, activity level and body weight goals [23]. The lower energy recommendation of 25 kcal/kg should be recommended for overweight and obese subjects.

Protein intake was higher than recommended in 67% of participants and consisted mostly of animal protein. A protein intake of 0.6–0.8 g/kg has been recommended in CKD pre-dialysis participants; levels of 0.8 g/kg has been found to prevent a negative nitrogen balance together with a sufficient energy intake [32]. The most recent KDOQI guidelines suggest a protein intake of 0.55–0.6g/kg per day [23]. The high protein intake in most participants in this group differs from other studies, where intakes were reported to be mainly lower than recommended. A high protein intake is associated with a more rapid decline in kidney function and other complications [33]. This high intake of protein could also be reflective of the higher energy intake in some participants.

Protein intake did not differ in the GFR categories but was found to be significantly higher in participants with a higher income. Protein is often the most expensive food item when shopping, with processed protein sources being cheaper. The cost of food was found to be a barrier to purchasing healthy food options in this study. Although the average income was low, the animal protein intake was surprisingly high in this study. A low income increases the risk of disorders that predispose CKD progression and worsens outcomes in those who already have CKD [34]. Socio-economic factors are important to consider in CKD management. A majority of participants did not complete high school, a lower level of education has been associated with decreased adherence behavior in CKD due to lower health literacy [34].

The high animal protein intake could explain why dietary saturated fat intake and cholesterol intake were higher than recommended ranges, as well as why the total and LDL cholesterol levels were raised. Few participants were using statins. Although total fat is within the recommended ranges, the intake of healthier fats such as polyunsaturated and monounsaturated fat should be favored. These intakes were lower than recommendations for most participants.

Although the fiber intake was low for the most participants, the mean intake was good and marginally higher than reported in other studies [1,29]. Fiber intake is usually low in CKD participants due to dietary restrictions of wholegrains, fruit and vegetables. A high fiber intake has recently been found to reduce uraemic toxins and subsequently improve the gut microbiome in CKD [35]. Fiber regulates the bacteria in the gut and enhances the growth of saccharolytic bacteria [36]. These are essential as fuel cells for the colonic epithelial cells and regulatory T lymphocytes. These cells are already reduced in renal failure and this, together with a low fiber diet, may account for CKD-associated systemic inflammation [37]. Inflammation was high in this study, and it is usually associated with adverse outcomes such as increased mortality, progression of disease, increased cardiovascular disease, muscle wasting and cognitive decline [38].

Participants in this study had a very high sugar intake. Excessive intake of refined sugar can increase triglycerides and contribute to obesity. A recent review of refined sugars in CKD indicates that it is a driver of kidney disease and its consequences by causing metabolic derangements such as insulin resistance and uric acid production. This increases the conversion of glucose to fructose via the polyol pathway; this pathway has recently been implicated to cause kidney damage [39].

Phosphate intake was high in a larger percentage of participants, and calcium and vitamin D levels were low. Vitamin D intake is generally low in the general population and even more so in CKD

participants [40]. A high phosphate intake was found in nearly half of the participants. High phosphate levels have been associated with increased cardiovascular disease due to vascular calcification as well as increased mortality [41]. Mineral and vitamin intake also varied. The low intake of folate and vitamin B6 intake are concerning since they are co-factors of homocysteine metabolism. Hyperhomocystenemia can contribute to cardiovascular disease, which is already a high risk in CKD participants [42]. Only a few participants were on a folate supplement and folate intake was lower than recommendations in all participants. Most participants had an adequate intake of other vitamins and minerals.

Limitations of Study

There are various dietary intake assessment tools including 24-h recalls, food records and food frequency questionnaires, which are each subject to strengths and limitations. Although highly accurate data can be obtained with a food frequency questionnaire, measurement errors related to the methodology remain [43]. The relatively small sample size and strict exclusion criteria may have limited the inclusion of older participants. For muscle mass assessment, bio-electrical impedance measures and 24-h urine creatinine excretion may have given more accurate results, however this was not done due to resource limitations. Hypertension as a cause of CKD has been challenged, however we relied on the diagnosis that clinicians had recorded in the medical notes. Without renal biopsies being documented, it may be that hypertension was not the most prevalent cause of CKD.

5. Conclusions

This study set out to determine the nutritional status of CKD patients and found a variety of factors, both from a medical and social perspective predisposed this younger study population to CKD development and increased risk of cardiovascular disease. These factors include hypertension, inflammation, obesity, dietary, socioeconomic and education factors.

Obesity was highly prevalent, with a low prevalence of undernutrition. Dietary evaluation showed an unhealthy Western dietary pattern. The standard nutrition assessment methods gave a good overall impression of the nutritional status of patients.

Nutrition advice should also aim to improve the diet to a healthier pattern and target nutrition supplementation such as folate, calcium and vitamin D with more emphasis on obesity management such as stricter dietary recommendations, physical activity guidelines and behavior modification for weight loss. Medical and surgical options should be considered to treat more advanced obesity. A multi-faceted approach should be employed to deal with the disparities that predispose the population to the development of CKD as well as improving outcomes in those with CKD. On a population wide level, efforts should be employed to reduce the high prevalence of obesity, hypertension and other chronic conditions to prevent the development of CKD.

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Appendix A

Table A1. Anthropometric Measurements and Interpretations.

Measurement	Formula	Cut-Off Values	Interpretation
BMI [17]	Weight/height^2	<18.49 kg/m^2	underweight
		18.5–24.99 kg/m^2	normal weight
		25–29.99 kg/m^2	overweight
		>30 kg/m^2	obese
Adjusted body weight [44]	$aBW_{ef} = BW_{ef} + [(SBW - BW_{ef}) \times 0.25]$ ef: oedema free weight		
MUAC [45]		<23 cm females <22 cm males >28 females >29 males >30 females and males	Malnourished Malnourished Overweight Overweight Obese
WC [46]		<80 cm for females <94 cm for males	Normal
		between 80–88 cm for females between 94–102cm for males	Increased risk for disease
		>88 cm for females and >102 cm for males	High risk for disease
AFA/AMA area [44]	AFA = [MAC(cm) × TSF(cm)/2 π × TSF(cm)^2]/4 π AMA = [MAC (cm) − (π × Triceps Skinfold Thickness (cm))]2/4 π	<5th percentile	Wasted
		≥5th and ≤15th percentile	Below average muscle/fat
		≥15th and ≤85th	Average muscle/fat
		≥85th and ≤ 95th percentile	Above average muscle/fat

BMI: Body mass index; MUAC: Mid upper arm circumference; WC: Waist circumference; AFA: Arm fat area; AMA: Arm muscle area.

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Effect of simplified dietary advice on nutritional status and uremic toxins in chronic kidney disease participants

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ARTICLE

Effect of simplified dietary advice on nutritional status and uremic toxins in chronic kidney disease participants

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Background: Traditional chronic kidney disease (CKD) dietary advice is challenging with many restrictions, consequently adherence to the CKD diet is low. Recent literature has proposed less restrictive dietary guidelines in CKD to improve dietary adherence and outcomes; however, limited evidence of its implementation exists.
Objectives: This study (trial number: PACTR202002892187265) investigated the effect of simplified dietary advice on nutritional outcomes and adherence after four weeks of dietary advice.
Design: A before-and-after study was conducted.
Outcome measures: Sociodemographic, clinical and biochemical information was collected and anthropometric measurements performed on Stage 3–5 CKD participants attending a pre-dialysis clinic. Uremic toxins were quantified by UPLC/fluorescence detection. Dietary intake was assessed using a quantified food frequency questionnaire (QFFQ). Participants were educated by the study dietitian on simplified dietary advice using an infographic. A diet-adherence score sheet monitored adherence. All outcomes were measured at baseline and four weeks after the diet was advised. IBM SPSS® version 27 was used for statistical analysis.
Results: Fifty-nine participants, mean age 41.0 ± 11.6 years, completed the study. After four weeks, significant improvements were found in body mass index ($p < 0.006$), waist circumference ($p < 0.001$), mid-upper arm circumference ($P < 0.001$), serum total cholesterol ($p < 0.045$), serum triglycerides ($p < 0.017$), energy ($p < 0.001$), protein ($p< 0.001$) and most dietary intake variables. Overweight and obesity prevalence was high at 68%. Uremic toxin concentrations remained stable. Dietary adherence was 88.6%.
Conclusion: The simplified dietary advice suggests improved nutritional outcomes in CKD patients who were predominantly overweight and obese, without compromising kidney function. This study highlights the importance and feasibility of simplified nutrition education in CKD.

Keywords: dietary adherence in CKD, infographic, nutrition education in CKD, simplified CKD advice, uremic toxins

Background

Chronic kidney disease (CKD) is highly prevalent globally and in sub-Saharan Africa.[1–3] Globally, there has been an increase in CKD morbidity, mortality and disability-adjusted years of life, with an increased burden of CKD in sub-Saharan Africa.[3] There are many complications related to CKD including anemia, malnutrition, anorexia, mineral and bone disease, electrolyte disturbances, cardiovascular disease and progression to end-stage kidney disease (ESKD).[4] In addition, patients with CKD may have several co-morbidities including obesity, hypertension and diabetes.[5] Traditional CKD nutritional advice has been challenging to convey to patients owing to the complexity of the diet. Patients had been advised to restrict fruits, vegetables, legumes, wholegrains, dairy and nuts owing to their phosphate and potassium content.[6] In addition, protein restrictions are needed to mitigate deterioration of kidney function; these factors and disease-related symptoms such as nausea, vomiting and anorexia result in low adherence to dietary advice.[7] To ensure sound nutritional advice and to improve adherence, these and additional factors including side effects of medications, financial constraints and dietary acceptance should be considered by healthcare professionals.

Dietary practice guidelines that formulated the traditional CKD advice originated from the Kidney Disease Quality Initiative (KDOQI) as well as the Kidney Disease Improving Global Outcomes (KDIGO).[8,9] These guidelines were partly evidence-based and partly based on expert opinion, or extrapolated from research on individuals without CKD.[10] Limited clinical trials investigating nutritional interventions were reported. Nutritional recommendations were mainly driven by the clinical judgement of physicians and dietitians rather than graded scientific evidence; the trials that were included had small sample sizes and primarily focused on one or two aspects of the diet.[10] The CKD nutrition guidelines have recently been updated and include more robust evidence for nutritional recommendations, including advice on dietary patterns, with less emphasis on specific nutrients.[11]

Recent studies have suggested that CKD dietary advice should be simplified to improve adherence.[6,12] Dietary pattern studies have shown that Western diets high in fat, sugar and energy are associated with increased mortality in CKD, whereas dietary patterns that reflect a healthier diet show improved outcomes in CKD.[13,14] The KDOQI 2020 updated dietary guidelines suggest that there is sufficient evidence that diets rich in fruits, vegetables, lean meats, low-fat dairy and low salt improves clinical outcomes, notably mortality.[11]

The current focus of CKD nutritional management should therefore be on natural, healthy foods with the exclusion of processed and 'fast' foods and foods high in salt and sugar

content. This will allow for the dietary goals for CKD to be met, while still permitting the consumption of a greater variety of foods.

Uremic toxins are retained in CKD, which enhances cardiovascular morbidity and mortality and the progression to ESKD.[15] Several of these uremic toxins are intestinally generated by the gut microbiota and are increased when there is gut dysbiosis.[15] Low-fibre diets and prolonged colonic transit time can contribute to this dysbiosis by reducing saccharolytic bacteria and favouring proteolytic bacteria.[16] The uremic toxins *p*-cresyl sulfate (*p*CS), *p*-cresyl glucuronide (*p*CG), indoxyl sulfate (IxS) and indole-acetic acid (IAA) have been shown to interact negatively with biological functions and to affect CKD progression, with levels being much higher in CKD patients than in healthy individuals. Prebiotic and probiotic supplementation has shown a reduction in uremic toxin levels and improved kidney function in CKD.[17–20] While these studies focus on prebiotics, probiotics or synbiotics, there are no studies of which we are aware, investigating CKD diet interventions only on uremic toxins in adults with CKD.

A previously developed simplified CKD dietary infographic based on a review of the scientific evidence was used to educate participants.[21] In this sub-study we aimed to investigate the effect of simplified dietary advice on nutritional outcomes and dietary adherence. Adherence to the diet would allow dietary changes to be made during the run-in period before the main trial commenced. This was investigated by educating participants on the dietary advice, and thereafter measuring outcomes such as anthropometry, kidney function, dietary intake and adherence, clinical factors, biochemical values and uremic toxin levels at baseline and after four weeks.

Subject and methods

Ethics and informed consent

The study was conducted in accordance with the Declaration of Helsinki. Ethics approval was obtained from the Human Research Ethics Committee at Stellenbosch University (reference number: S18/03/064) and participants were enrolled after written informed consent was obtained. Participants were anonymized on datasets for analysis.

Study design, sampling and participants

This nested study formed part of a randomised controlled trial (RCT) (trial number: Pan African Trial Registry: PACTR202002892187265), investigating the effect of a prebiotic on uremic toxins, CKD outcomes and the gut microbiome. Participants were enrolled at their routine doctor's appointment at a pre-dialysis clinic in Cape Town, South Africa, provided they were older than 18 years and presented with stage 3–5 CKD. This before and after sub-study investigated the effect of simplified dietary advice on the nutritional status, uremic toxins and dietary adherence of CKD participants, representing the run-in period of the main trial. A control group was not assigned owing to all of the participants having to follow the diet during the run-in period. All participants were educated and placed on the simplified dietary regime and outcomes were measured before and after four weeks of the dietary advice. Participants were excluded if they met the following criteria: taking antibiotics, prebiotics or probiotics, active gastrointestinal conditions (such as severe diarrhoea, constipation and abdominal cramping, inflammatory bowel disease or Crohn's disease), malignant hypertension, crescentic glomerular nephritis, diabetes, coeliac disease or any infectious diseases that would affect nutritional status. Measurements were performed at enrolment (Baseline) and after four weeks (Week 4).

Sample size

The sample size was calculated for the main clinical trial using a two-sample t-test using the Power Analysis Sample Size software (PASS program) based on previous study outcomes.[22] A power of 90% was used. The total number of participants needed for the main trial was 46, but, owing to expected attrition, 70 participants were enrolled. Eleven participants were lost to follow-up.

Diet advice and assessment

The study dietitian provided individual dietary counselling based on CKD guidelines to all participants using simplified advice.[8,9] The older guidelines were used because the updated KDOQI guidelines were only released after the study was conducted. Therefore, protein was restricted to 0.8 g/kg and no specific energy guidelines were given. All participants also received the following simplified key guidelines that were part of the infographic: limiting of additives, processed foods, salt and salty foods, high-phosphate meats and encouraging low-fat proteins, wholegrains and an adequate intake of fruits and vegetables. Additional tips on cooking, alcohol and fluid intake were included. These guidelines were conveyed to the participants as an infographic, which was developed based on scientific evidence, together with predetermined protein exchanges.[21] At the end of the study the participants were asked about the ease of use of the infographic and whether it assisted them in making dietary changes.

A 160-item interviewer-administered quantified food frequency questionnaire (QFFQ) was adapted from a previous QFFQ[23] to assess dietary intake at both visits. The QFFQ was adapted for suitability in CKD to include a wider variety of potassium-, sodium- and phosphate-containing foods. The following foods were added to the original QFFQ: a wider variety of fruits and vegetables for their potassium content, processed and crumbed protein containing foods and dark cold drinks for their phosphate content, as well as added salt for sodium content. The QFFQ contained food items from various food groups so that energy, macronutrients and micronutrients could be analysed. The QFFQ was tested for face validity with patients with CKD and content validity with specialist dietitians and changes made accordingly, before being finalised. Household food utensils and food models were used to estimate portion sizes. The recorded food portions were quantified using a food quantities manual and entered into a database, coded and calculated to grams per daily intake. Dietary intake was analysed for its nutrient content by a statistician from the South African Medical Research Council using the SAFOODS Database.[24] An adapted dietary adherence score sheet was also developed to determine whether participants were adhering to the diet and was completed at Week 4. The adherence score sheet was adapted for CKD from the PREDIMED adherence score sheet.[25] There were 12 questions on dietary changes from various food groups with a set of criteria (Appendix A). The criteria were selected based on information advised in the infographic and the dietary advice given. These included five questions on adhering to protein allowance, sufficient fruit and vegetable intake, healthy cooking methods and wholegrain intake, seven questions on the avoidance of processed food high in additives including fizzy cool drinks, 'fast' foods, alcohol intake, salt and salty foods and high-phosphate

meats. A score of 1 was allocated if they were adhering to the criteria. The scores were totalled, and adherence was calculated as a percentage out of 12.

Anthropometric assessments

Anthropometric measurements included: weight and height, waist circumference, triceps skinfold and mid-upper arm circumference (MUAC) using standard measuring techniques.[26] A calibrated Seca scale (Seca GmbH, Hamburg, Germany) was used to measure weight, while a Seca stadiometer was used to measure height, a plastic measuring tape was used for the waist circumference and MUAC and a Harpenden Caliper (Baty International, Burgess Hill, UK) was used to measure triceps skinfold. Three measurements were performed, and the average was recorded. The body mass index (BMI) was calculated by dividing the weight by the height squared. The BMI and waist circumference were interpreted according to World Health Organization (WHO) guidelines.[27] Mid-upper arm circumference was interpreted using standard measures.[28] Triceps skinfold was recorded.

Biochemistry and uremic toxin assessments

Routine blood results such as kidney function and electrolytes were obtained from clinic records and blood was drawn by the clinic nurse for additional biochemistry such a full lipid profile, uremic toxins and C-reactive protein (CRP) and analysed by the National Health Services (NHLS) laboratory. The blood samples for uremic toxin analysis were delivered directly to the NHLS laboratory onsite for further analysis.

For the determination of uremic toxins, venous blood was collected at baseline and Week 4 in K-EDTA tubes (9 ml). Blood was immediately centrifuged at 2100 × g for 10 minutes at 4°C. Plasma was aliquoted on ice at 500 µl in sterile tubes. Plasma was stored at −80°C. The NHLS laboratories were used to store the blood samples for uremic toxins analysis. Plasma was sent on dry ice to the Nephrology Laboratory of the Ghent University Hospital in Belgium for batch analysis. Liquid chromatography and fluorescence detection determined total and free concentrations of *p*CS, IxS, *p*CG and IAA as previously described.[29] In brief, plasma samples were deproteinized by heat, centrifuged and filtered through an Amicon Ultra 0.5 µl (Merck, Merck Millipore Ltd. Tullagreen, County Cork, Carrigtwohill, Ireland) (molecular weight cut-off off 30 kDa Filters) for the quantification of the total toxin concentrations. For the free
fraction quantification, Amicon filters were used to first filter the untreated plasma. The ultrafiltrate was transferred into an autosampler vial, and fluorescein was added as an internal standard. Analysis was performed by ultra-performance liquid chromatography with an Agilent 1290 Infinity device (Agilent, Santa Clara, CA, USA): IxS (λex: 280 nm, λem: 376 nm), *p*CS and *p*CG (λex: 264 nm, λem: 290 nm), IAA (λex: 280 nm, λem: 350 nm). Fluorescein (λex: 443 nm, λem: 512 nm) was detected by an Agilent G1316C fluorescence detector.

Clinical measurements

Blood pressure was measured by the clinic nurse and recorded. Ankle oedema was assessed by the dietitian by applying pressure on the tibia of the ankle with thumb and releasing after five seconds. The oedema was graded according to the time it took to rebound.[30] The severity was based on a Likert scale from 1 to 4 with one being no oedema and four being severe oedema.

Statistical analyses

IBM SPSS® version 27 (IBM Corp, Armonk, NY, USA) was used for statistical analysis. Data were checked for normality using Shapiro–Wilk, histograms and skewness values. Means were reported for normally distributed data and medians and interquartile ranges for non-normal distributed data. Frequencies were reported for categorical data. Normally distributed data were compared from Baseline to Week 4 using paired *t*-tests, while non-normal data were compared using Wilcoxon tests. Categorical variables were compared using Mcnemar tests. A *p*-value of < 0.05 was considered significant.

Results

Sociodemographics

Fifty-nine participants completed the study. The sociodemographic information is presented in Table 1. This predominantly female group (57.6%) were fairly young (41 ± 11.6 years). Half of the participants were employed, and the income earned was less than US$126 per month for most participants. The main cause of kidney failure as documented in the medical files was hypertension, and most participants were in stage 5 CKD.

Dietary intake

Table 2 shows the comparison of the dietary intake between Baseline and Week 4. There were highly significant reductions in all macronutrients and micronutrients including potassium, phosphate and sodium between the two visits, except for total sugar.

Anthropometry

A significant decrease occurred in the weight ($p < 0.006$), BMI ($p < 0.006$), MUAC ($p = 0.001$) and waist circumference ($p < 0.001$) over the four-week period as shown in Table 3. Although these were small differences, the maximum loss in weight was 3.5 kg and 13.3 cm in waist circumference. Of note was that some participants' weights remained stable (11.9%), most lost weight (68%) and a few gained weight (18.6%). A majority of participants (68%) were overweight and obese (Figure 1) and abdominal obesity was present in 59% of participants at baseline.

Table 1: Sociodemographic data of study participants ($n = 59$)

Item	Factor	*n* (%)
Age (years) (Mean ± SD)		41.0 ± 11.6
Gender	Male	25 (42.4)
	Female	34 (57.6)
Income (per month)	$0–$126	24 (40.7)
	$127–$316	16 (27.1)
	$317–$633	13 (22.0)
	$634–$949	4 (6.8)
	> $949	2 (3.4)
Employment	Employed	29 (49.2)
	Unemployed	30 (50.8)
Cause of kidney failure	Polycystic kidneys	3 (5.1)
	Hypertension	29 (49.2)
	Glomerular disease	13 (22.0)
	Other	14 (23.7)
GFR categories (ml/min/1.73 m²)	30–59	19 (32.2)
	15–29	16 (27.1)
	< 15	24 (40.7)

Abbreviations: GFR = glomerular filtration rate.

Table 2: Dietary intake changes between Baseline and Week 4

Nutrients	Baseline Mean ± SD Median (IQR) *n* = 59	Week 4 Mean ± SD Median (IQR) *n* = 59	*p*-value
Energy (kcal/kg) Total kJ	27 8 018.2 (6 101.0, 10 114.0)	19 5 710.0 (4 480.0, 6 982.0)	< 0.001
Total protein (g/kg) Total protein (g)	1.0 72.5 ± 26.9	0.7 51.9 ± 20.5	*< 0.001
Plant protein (g)	24.7 (17.0, 30.1)	16.6 (14.0, 21.1)	< 0.001
Animal protein (g)	43.9 (32.0, 55.0)	31.6 (22.4, 39.6)	< 0.001
Total fat (g)	74.7 (53.0, 100.3)	50.0 (35.1, 60.8)	< 0.001
Saturated fat (g)	22.8 (14.9, 31.0)	13.05 (9.6, 18.8)	< 0.001
Total trans-fat (g)	0.5 (0.3, 0.9)	0.3 (0.1, 0.6)	0.001
Cholesterol (mg)	249.8 (167.6, 345.2)	148.0 (114.0, 228.5)	< 0.001
Carbohydrate (g)	244.7 ± 90.3	186.0 ± 68.9	*< 0.001
Added sugar (g)	37.4 (22.4, 56.0)	27.5 (13.7, 44.5)	< 0.001
Total sugars (g)	69.0 (49.0, 81.9)	60.0 (47.0, 76.0)	0.097
Dietary fibre (g)	19.4 (13.8, 28.5)	17.6 (14.1, 21.3)	0.005
Phosphate (mg)	942.2 (668.9, 1229.5)	735.6 (523.0, 939.7)	< 0.001
Sodium (mg)	1 829.2 (1 290.4, 2 584.8)	1 037.0 (673.5, 1 445.0)	< 0.001
Potassium (mg)	2 525.9 (1 942.7, 3 304.6)	1 923.0 (1 553.5, 2 405.4)	< 0.001

Statistical tests: Wilcoxon tests, *paired *t*-tests. Bold if $p < 0.05$.

Biochemistry

There were significant reductions in serum sodium ($p < 0.001$), serum total cholesterol ($p = 0.045$) and serum triglycerides ($p = 0.017$), while serum potassium slightly increased ($p = 0.046$) from Baseline to Week 4 as shown in Table 4. Serum urea, creatinine and phosphate tended to decrease. The plasma levels of the uremic toxins remained stable between Baseline and Week 4. There were 4 missing samples at Week 4, therefore only 55 samples were analysed.

Clinical changes

Most of the participants had no oedema Table 5. There was a significant reduction in the moderate and severe categories with more participants shifting to the mild category at week 4. Blood pressure did not change significantly.

Dietary adherence scores

Participants reported overall adherence scores of 88.6% to the dietary guidelines advised. Of the individual adherence score categories, only 47.5% of participants adhered to the fruit criteria of 2–3 servings of fruit a day, 69.5% to the vegetable criteria of 2–4 servings a day and 81.5% of participants to the wholegrain servings criteria of more than 2 servings a day. The salt and processed food category as well as the other food categories were adhered to in more than 90% of participants (Figure 2).

Table 3: Anthropometrical changes between Baseline and Week 4

Measurement	Baseline Mean ± SD *n* = 59	Week 4 Mean ± SD *n* = 59	*p*-value
Weight (kg)	76.4 ± 21.1	75.7 ± 20.7	0.006
BMI (kg/m²)	28.6 ± 6.7	28.3 ± 6.6	0.006
Waist (cm)	91.5 ± 15.6	89.6 ± 15.0	< 0.001
MUAC (cm)	31.0 ± 5.5	30.2 ± 5.1	0.001
Triceps skinfold (mm)	21.4 ± 8.9	21.4 ± 9.0	0.913

Abbreviations: BMI = body mass index; MUAC = mid upper-arm circumference. Statistical tests used: paired *t*-tests. SD = standard deviation. Bold if $p < 0.05$.

Usefulness of infographic

All participants indicated they found the infographic useful, understood it and that it assisted them in making dietary changes.

Discussion

The simplified dietary advice suggests improved nutritional parameters and adherence to dietary guidelines over the short term.

Low-protein diets are effective in improving outcomes in CKD,[31] but dietary adherence remains poor. Dietary education for CKD is usually complex and time consuming, owing to multiple dietary restrictions[7] and particularly in South Africa there are extensive exchange lists for CKD complicating dietary education.[21] Additionally, in resource-limited settings, dietary education from dietitians is limited. Pisani *et al.*[32] reported promising improvements in metabolic parameters and dietary adherence by using simplified dietary guidelines in CKD.

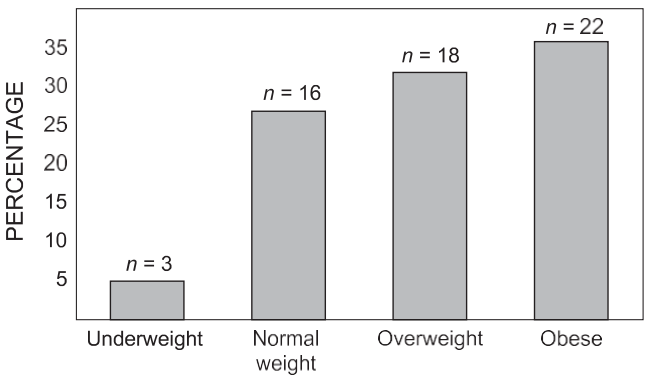

Figure 1: BMI categories of participants (%) at baseline.

Table 4 : Biochemical changes between Baseline and Week 4

Biochemical value	Normal ranges	Baseline Mean ± SD Median (IQR) *n* = 59	Week 4 Mean ± SD Median (IQR) *n* = 59	*p*-value
Urea (mmol/l)	2.1–7.1	14.4 (10.3, 25.0)	14.2 (9.1, 28.6)	0.775
Creatinine (umol/l)	64.0–104.0	269.0 (178.0, 447.0)	232.0 (175.0, 461.0)	0.804
GFR (ml/min.1.73m²)	> 60	20.0 (11.0, 35.0)	20.0 (11.0, 35.0)	0.822
Potassium (mmol/l)	3.5–5.1	4.8 ± 0.6	4.9 ± 0.7	*0.046
Sodium (mmol/l)	136.0–141.0	141.0 ± 2.9	139.3 ± 2.6	*< 0.001
Phosphate (mmol/l)	0.78–1.42	1.3 (1.1, 1.5)	1.16 (1.0, 1.5)	0.174
Total cholesterol (mmol/l)	< 4.5	4.9 ± 1.2	4.7 ± 1.1	*0.045
LDL (mmol/l)	< 2.5	2.7 ± 1.0	2.6 ± 1.0	*0.143
HDL (mmol/l)	> 1.2	1.1 (0.9, 1.4)	1.1 (0.9, 1.3)	0.055
TG (mmol/l)	< 1.7	1.9 (1.2, 2.6)	1.6 (1.2, 2.4)	0.017
CRP (mg/l)	< 10.0	5.0 (1.0, 9.0)	4.0 (2.0, 8.0)	0.329
Uremic toxins		*n* = 55	*n* = 55	
Total IxS (mg/l)	0.53	4.47 (1.90, 8.47)	3.96 (1.55, 10.27)	0.560
Free IxS (mg/l)	ND	0.10 (0.05, 0.29)	0.09 (0.03, 0.27)	0.131
Total *p*CS (mg/l)	1.90	5.77 (3.02, 9.84)	5.69 (2.86, 10.37)	0.597
Free *p*CS (mg/l)	0.08	0.14 (0.06, 0.25)	0.14 (0.06, 0.30)	0.267
Total *p*CG (mg/l)	–	0.09 (0.03, 0.23)	0.11 (0.03, 0.26)	0.199
Free *p*CG (mg/l)	–	0.09 (0.02, 0.18)	0.09 (0.02, 0.20)	0.506
Total IAA (mg/l)	0.50	0.70 (0.49, 1.55)	0.80 (0.50, 1.33)	0.855
Free IAA (mg/l)	–	0.13 (0.09, 0.27)	0.12 (0.08, 0.25)	0.913

Abbreviations: GFR = glomerular filtration rate, LDL = low-density cholesterol, HDL = high-density cholesterol, TG = triglycerides, CRP = C-reactive protein, ND = not detectable; IxS = indoxyl sulfate, *p*CS = *p*-cresyl sulfate, *p*CG = *p*-cresyl glucuronide, IAA = indole-3-acetic acid. Statistical tests: Wilcoxon tests,*paired *t*-tests. Bold if $p < 0.05$.

Dietary pattern studies show that whole foods focusing on a prudent diet rich in fish, poultry, fruit, vegetables and legumes showed no association with albuminuria, while GFR remained unchanged compared with those consuming a more Westernised diet rich in processed foods, sugar and salt, resulting in microalbuminuria and a decline in the glomerular filtration rate (GFR).[14] Similarly, the current study also showed a stable GFR by following simplified dietary advice. In addition, there was an improvement in other nutrition outcomes such as anthropometry, dietary intake and lipid values, while uremic toxins also remained stable.

The usefulness of the infographic reported by the participants may explain why the dietary adherence was high; this is much higher than reported in other studies, although this may be owing to the shorter duration of the present study. It could also be owing to the individual counselling the participants received at the start of the study. Studies have shown that patients with CKD have an adherence of 70% with intensive counselling compared with a 48% adherence with standard counselling.[33] This shows that more intensive counselling has a positive effect on dietary adherence. The improvement in the adherence in this study may therefore relate to the simplified nature of the dietary education allowing more variety, as well as the individual counselling participants received. However, in resource-limited settings, individual counselling is not always available. The simplified infographic may still have some benefits in this instance, as it is easy to follow.

There was a significant decline in protein intake in the four-week period, from 1 g to 0. 7 g/kg of ideal bodyweight. This is within the KDOQI recommended guidelines of 0.6–0.8 g in pre-dialysis patients.[8] However, this guideline has recently been revised and the current recommendation is 0.55–0.6 g/ kg per day.[11] Low-protein diets reduce the progression of CKD.[4] The six tips diet intervention study used simplified

Table 5: Clinical changes between Baseline and Week 4

Clinical Factor		Baseline Median (IQR) *n* = 55	Week 4 Median (IQR) *n* = 55	*p*-value
Systolic blood pressure (mmHg)		141.0 (130.0, 159.0)	142.0 (128.3, 168.3)	*0.336
Diastolic blood pressure (mmHg)		79.0 (70.0, 89.0)	80.0 (72.0, 92.8)	*0.205
		Baseline *n* (%) *n* = 59	Week 4 *n* (%) *n* = 59	*p*-value
Oedema	None	35 (59.3)	37 (62.7)	0.008
	Mild	14 (23.7)	18 (30.5)	
	Moderate	7 (11.9)	3 (5.1)	
	Severe	3 (5.1)	1 (1.7)	

Statistical tests: *Wilcoxon and Mcnemar tests. Bold if $p < 0.05$.

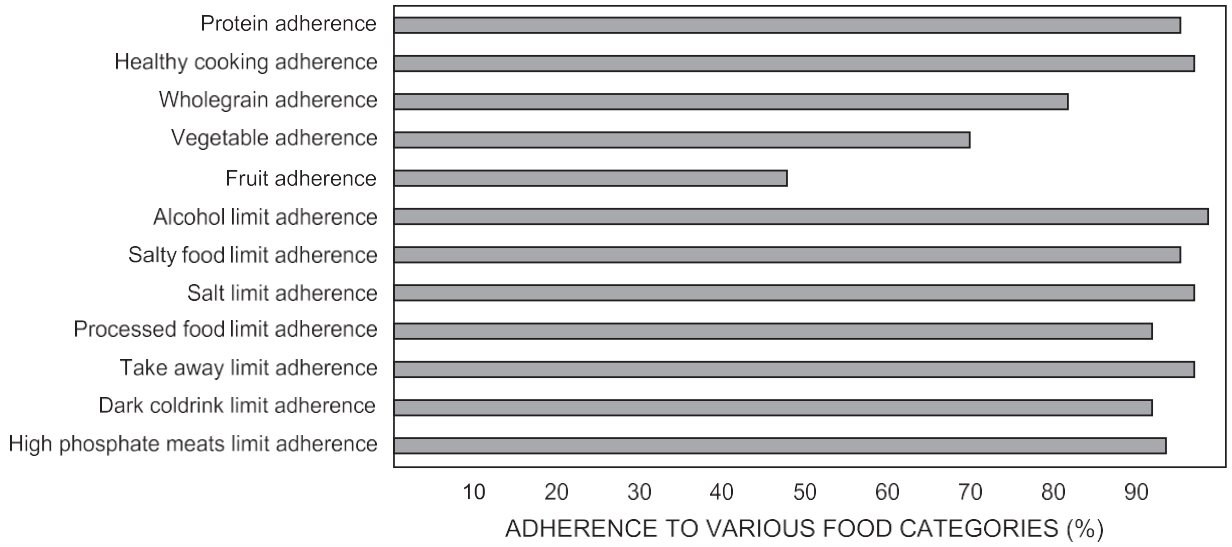

Figure 2: Adherence to various food categories (%).

written dietary suggestions (6TD) versus a standard low-protein diet (LPD) to improve dietary adherence in CKD patients over six months with follow-up at one, three and six months.[32] They showed a significant reduction in protein intake after three and six months in the 6TD group, with a reduction in serum urea only at six months in the 6TD group. This could explain why in the present study no difference in serum urea levels was observed after four weeks. The stabilising of kidney function in the present study is similar to the findings of Morales *et al.*,[34] who found no significant difference in GFR in patients on a weight-loss diet despite losing weight, whereas it declined by 8% in the control group.

A meta-analysis on the effect of weight-loss interventions on kidney outcomes in non-surgical weight-loss patients suggests that weight loss does not seem to affect GFR.[35] Although weight loss was not one of the goals of this present study, there was a significant although small reduction in the mean weight, BMI and waist circumference in participants, and most participants lost weight. Although participants were not advised on a specific energy allowance, there was a significant reduction in energy intake and all other macronutrients, which may explain the weight reduction. The weight loss is beneficial especially in this group where overweight and obesity prevalence was very high. The prevalence of obesity in this population has been reported[36,37] and has been associated with negative outcomes.[5] Weight should be monitored over a longer duration to see if the effect of weight loss will be sustained. Since malnutrition has commonly been reported in the CKD population,[38] caution should be advised in undernourished patients not to reduce overall energy intake drastically while following simplified dietary advice to prevent further weight loss. Reassuringly, in the current study the underweight participants did not lose weight by following the simplified guidelines.

The present study had a reduction in all fats, carbohydrates and sugar intake from Baseline to Week 4, which may have resulted in the significant effects on lipid values. Other studies do not report on all the dietary analyses from baseline to the intervention period, making comparisons difficult. Although the effect of saturated fat increasing low-density lipoprotein (LDL) has mainly been studied, the results are ambiguous. In a review comparing the relationship of sugar and saturated fat's effect on cardiovascular disease, it was shown that diets should focus on the elimination of refined sugar, rather than advising on reducing saturated fat to reduce cardiovascular disease risk.[39] A high sugar intake increases uric acid and insulin resistance. This increases the conversion of glucose to fructose through the polyol pathway; this pathway has been implicated as contributing to CKD progression.[40] Nonetheless, total fat and favouring plant-based unsaturated fat should still be important components of CKD dietary advice.

As expected, the concentrations of the uremic toxins were higher than the normal ranges for healthy individuals.[41] However, there were no significant differences in uremic toxins concentrations from Baseline to Week 4. This finding may in itself be a positive finding as the uremic toxins stabilised and did not increase. Although the diet changed significantly in most nutrients, the fibre intake was marginally reduced, which may have influenced this result. Fibre is important to maintain a healthy gut microbiome, enhancing saccharolytic bacteria and reducing proteolytic bacteria, which are mainly responsible for the production of the precursors of uremic toxins.[42] Although participants were advised to increase wholegrains, fruits and vegetables, the fibre intake did not increase. This may relate to the overall reduction in energy between baseline and Week 4. The fruit and vegetable intake categories were adhered to by fewer participants in the present study according to the adherence score sheet, with fruit intake adherence being the lowest. Socioeconomic status may have contributed to low fruit and vegetable intake, because half of the participants were unemployed, and most were in the low-income range. Low fruit and vegetable intake has been found in socioeconomically disadvantaged communities in South Africa.[43] In addition, it was reported that a third of South Africans consume two or fewer portions of fruit and vegetable per day.[44] Participants may also have been hesitant to increase their fruit and vegetable intake owing to potassium limitations advised on the traditional CKD diet in the past.

While dietary protein intake dropped significantly in the present study there was only a trend for a decrease in levels of total and free IxS. Other studies have shown a reduction in IxS levels on a very low-protein diet of 0.3 mg/kg supplemented with keto-analogues,[45] compared with the present study which was at 0.7 g/kg. Guida *et al.*[20] reported an increasing trend in *p*CS over a one-month period in their control group (CKD 3–4)

compared with their intervention group taking a synbiotic in haemodialysis patients, in which *p*CS was significantly reduced. It seems that dietary supplements such as prebiotics, probiotics or synbiotics may be necessary to reduce uremic toxins significantly, whereas the diet seems to stabilise it. A cross-sectional dietary pattern study evaluating plant-based diet quality with uremic toxins and gut microbiota in haemodialysis patients showed that the quality of the diet, particularly plant-based foods, either suppressed or promoted certain microbes, which in turn links to the concentrations of uremic toxins.[46] Ultimately the quality of the diet affects the generation of gut-derived uremic toxins.[46]

Limitations of the study

This study did not have a control group because the study represented the baseline dietary education run-in period before the main trial. However, participants were compared with their baseline values before and after the simplified dietary advice was given, hence serving as their own controls. The assessment of fluid status is a subjective measure, and this may have impacted on the accuracy of the adjusted bodyweight. The dietitian was, however, standardised in these measurements, thereby minimising error. This study was also of a short duration of four weeks and did not impact on variables that need a longer time to change, such as kidney function. Highly accurate information can be obtained with an QFFQ; however, methodological flaws remain.[47] The adherence score sheet revealed high adherence in the short term; this also aligns with what was reported on the QFFQ, but there may be under-reporting involved with both the QFFQ and the adherence score sheet.

Recommendations

Randomised controlled trials investigating the effect of simplified dietary advice using a longer study period should be performed in CKD pre-dialysis patients. Dietetic counselling and education should be offered to all pre-dialysis CKD patients, rather using simplified advice with some graphics to explain the diet, focusing on the whole diet. A concerted effort to increase dietary fibre intake should be considered in future intervention studies to at least recommended levels, possibly by providing supermarket vouchers to purchase fruit and vegetables for those unable to afford these. Encouraging vegetable gardens may also be a solution. This would need to be considered at government level where policies are put in place to uplift all communities, not only specific to the CKD population.

Conclusion

In conclusion, the simplified dietary advice and counselling by the dietitian suggest favourable effects on many nutritional outcomes, including BMI, waist circumference, oedema, MUAC, dietary variables, serum cholesterol and triglycerides. Kidney function and uremic levels were stabilised over the four-week period. Diet adherence was high, allowing participants to improve their food choices. The dietitian is an integral part of the multidisciplinary team caring for CKD patients. This study emphasises the important and feasible role of simplified nutrition education in improving the nutritional status of CKD participants, especially in resource-limited settings.

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Appendix A

Question	Criteria for 1 point
1. Do you follow your protein allowance daily portions or with each main meal?	Yes
2. How many high-phosphate meats do you eat in a day, i.e. eggs, liver, kidney, cheese?	1
3. How many dark cold drinks do you consume in day?	< 1
4. How many takeaway foods do you consume in a week? E.g. burgers, chips, fried chicken	1
5. How many burgers, polonies, sausages, viennas, crumbed boxed items, packets of soups do you eat in a week?	< 2
6. How many pieces of fruit do you eat in a day?	2–4
7. How many vegetables do you eat in a day?	2–4
8. How many wholegrain foods do you eat in a day? E.g. wholewheat or brown bread or crackers, oats, all-bran, any other high-fibre cereal, wholewheat pasta	> 2
9. Do you add salt to your cooking (more than ¼ tsp per serving) or add at the table?	No
10. How many other salty foods do you eat in a day, e.g. salted chips, popcorn, packets of soups, sauces, biltong, readymade gravies	> 1
11. How many servings of alcohol do you have in a day?	< 1
12. Do you steam, grill, boil, braise your foods daily?	Yes

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CHAPTER 7: ARTICLE 4 (PUBLISHED)

The Effect of ß-Glucan Prebiotic on Kidney Function, Uremic Toxins and Gut Microbiome in Stage 3 to 5 Chronic Kidney Disease (CKD) Predialysis Participants: A Randomized Controlled Trial

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The Effect of ß-Glucan Prebiotic on Kidney Function, Uremic Toxins and Gut Microbiome in Stage 3 to 5 Chronic Kidney Disease (CKD) Predialysis Participants: A Randomized Controlled Trial

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Abstract: There is growing evidence that gut dysbiosis contributes to the progression of chronic kidney disease (CKD) owing to several mechanisms, including microbiota-derived uremic toxins, diet and immune-mediated factors. The aim of this study was to investigate the effect of a ß-glucan prebiotic on kidney function, uremic toxins and the gut microbiome in stage 3 to 5 CKD participants. Fifty-nine participants were randomized to either the ß-glucan prebiotic intervention group ($n = 30$) or the control group ($n = 29$). The primary outcomes were to assess kidney function (urea, creatinine and glomerular filtration rate), plasma levels of total and free levels of uremic toxins (*p*-cresyl sulfate (*p*CS), indoxyl-sulfate (IxS), *p*-cresyl glucuronide (*p*CG) and indoxyl 3-acetic acid (IAA) and gut microbiota using 16S rRNA sequencing at baseline, week 8 and week 14. The intervention group (age 40.6 ± 11.4 y) and the control group (age 41.3 ± 12.0 y) did not differ in age or any other socio-demographic variables at baseline. There were no significant changes in kidney function over 14 weeks. There was a significant reduction in uremic toxin levels at different time points, in free IxS at 8 weeks ($p = 0.003$) and 14 weeks ($p < 0.001$), free *p*CS ($p = 0.006$) at 14 weeks and total and free *p*CG ($p < 0.001$, $p < 0.001$, respectively) and at 14 weeks. There were no differences in relative abundances of genera between groups. Enterotyping revealed that the population consisted of only two of the four enterotypes: *Bacteroides* 2 and *Prevotella*. The redundancy analysis showed a few factors significantly affected the gut microbiome: these included triglyceride levels ($p < 0.001$), body mass index ($p = 0.002$), high- density lipoprotein ($p < 0.001$) and the prebiotic intervention ($p = 0.002$). The ß-glucan prebiotic significantly altered uremic toxin levels of intestinal origin and favorably affected the gut microbiome.

Keywords: chronic kidney disease (CKD); gut microbiome; uremic toxins; prebiotic

1. Introduction

Chronic kidney disease (CKD) prevalence is increasing globally with a prevalence of between 9 and 13% [1,2]. In sub-Saharan Africa, it is slightly higher at 14% [3], although this percentage may be underestimated owing to a lack of CKD statistics [4].

Cardiovascular disease (CVD) is the leading cause of mortality in CKD patients, despite medical treatment [5]. Reducing the progression of CKD is challenging and complex owing to the pathophysiology of the disease. The modulation of the gut microbiome has in recent years been suggested to be a therapeutic target in the management of CKD, because of its important role in kidney health [6–8].

The gut microbiome is home to trillions of cells of over 1000 species and is responsible for maintaining normal gut integrity and promoting immunological functions. It is involved with nutrient uptake and metabolism of nutrients, degradation of oxalates, and preventing the proliferation of harmful microorganisms [9,10]. It is considered to be an essential 'organ' due to its varied composition and roles in human health. In CKD, gut dysbiosis occurs because of gut wall edema, lack of fiber in the diet due to dietary restrictions, and use of antibiotics and phosphate binders, resulting in increased gut permeability [10,11]. Gut dysbiosis, in turn, contributes to uremic toxicity, inflammation and CVD [6,12–14]. This bidirectional relationship enhances the progression of CKD [6].

The gut-derived uremic toxins that are increased in CKD include indoxyl sulfate (IxS), *p*-cresyl sulfate (*p*CS), *p*-glucuronide (*p*CG), indole acetic acid (IAA) and trimethylamine-N-oxide (TMAO). They are associated with insulin resistance, increased oxidative stress and endothelial dysfunction [12,13,15]. The pro-inflammatory nature of these toxins results in the progression of CKD and increases mortality [14]. They originate in the colon, where aromatic amino acids are metabolized by bacteria into phenolic (*p*-cresol and phenol) and indolic compounds (indole and IAA) [16]. Phenolic compounds are produced by breakdown of tyrosine and phenylalanine, mainly by anaerobes, while indoles are produced by the bacterial breakdown of tryptophan by both aerobes and anaerobes [13].

A recent systematic review on the gut microbiome in CKD found differences in overall microbial diversity to be inconclusive between patients and healthy controls [17], but 20 microbial taxa were differentially abundant.

In general, gut microbiota can be stratified into four non-discreet community types, referred to as enterotypes [18]. The *Bacteroides* 2 enterotype, which is characterized by a large fraction of *Bacteroides* while having lower *Faecalibacterium* levels and overall microbial density, is known to be associated with inflammation [18,19]. Furthermore, *Bacteroide*s 2 has also been found to be associated with systemic inflammation linked with a high body mass index (BMI) [20] and mental health disease [21].

Pre-, pro- and synbiotics are potential therapeutic options to alter gut microbiota and reduce uremic toxin generation [22]. A systematic review reveals a low certainty of the effect of these agents owing to the varying results [22]. In addition, studies investigating the effect of prebiotics on kidney function, uremic toxins and the gut microbiome simultaneously are scarce.

Limited studies have investigated the use of prebiotics on the gut microbiome in CKD, making this an attractive therapeutic option to explore. ß-glucan, which is found in oats, seems promising to modulate the gut. Some studies in healthy individuals and those at risk of CVD showed that ß-glucan increased *Bifidobacterium* and *Lactobacillus*, reduced cholesterol levels and enhanced the production of short-chain fatty acids (SCFAs) [23,24] as well as reduced *p*CS in healthy individuals [25].

The aim of this study was to investigate the effect of ß-glucan on kidney function, uremic toxins and the gut microbiome in stage 3 to 5 CKD participants. The study hypothesis was that the prebiotic would improve kidney function, uremic toxin levels and the gut microbiome. This novel study is the first to investigate the effect of a prebiotic on all of these outcomes.

2. Materials and Methods

This randomized control trial (RCT) was a single-center, single-blinded study investigating the effects of a ß-glucan prebiotic on kidney function, plasma levels of uremic toxins and the gut microbiome. Participants were randomized to either receive a daily prebiotic supplement together with CKD dietary advice (intervention group) or to remain

on the diet only (control group). Ethics approval was obtained from the Health Research Ethics Committee of Stellenbosch University (S18/03/064), and the study adhered to the Declaration of Helsinki principles. The protocol submitted for ethics approval was adhered to during the study. Informed consent was obtained from participants before they enrolled in the study. The study was registered with the Pan African Trial Registry (PACTR202002892187265).

2.1. Participants

Participants (over 18 years) attending a predialysis clinic in Cape Town, South Africa, with CKD stage 3 to 5 (classified by a GFR< 60 mL/min per 1.73 m^2) were enrolled. Only participants over 18 years old were recruited. Participants were excluded if they met the following criteria: taking antibiotics, prebiotics or probiotic supplements currently or in the past four weeks, participants with inflammatory bowel disease, bowel malignancy, previous colorectal surgery (or any other serious bowel disorder), pregnancy, diabetes mellitus, coeliac disease, human immunodeficiency virus (HIV) disease, malignant hypertension, crescentic glomerular nephritis, participants on immunosuppressant medications, and those expected to start immediate dialysis. Figure 1 shows the CONSORT flow diagram for progress of participants through the trial. There were 108 participants eligible for inclusion, of whom 70 participants were enrolled for the pre-randomization period. Eleven were lost to follow-up at the baseline, with only 59 participants being randomized.

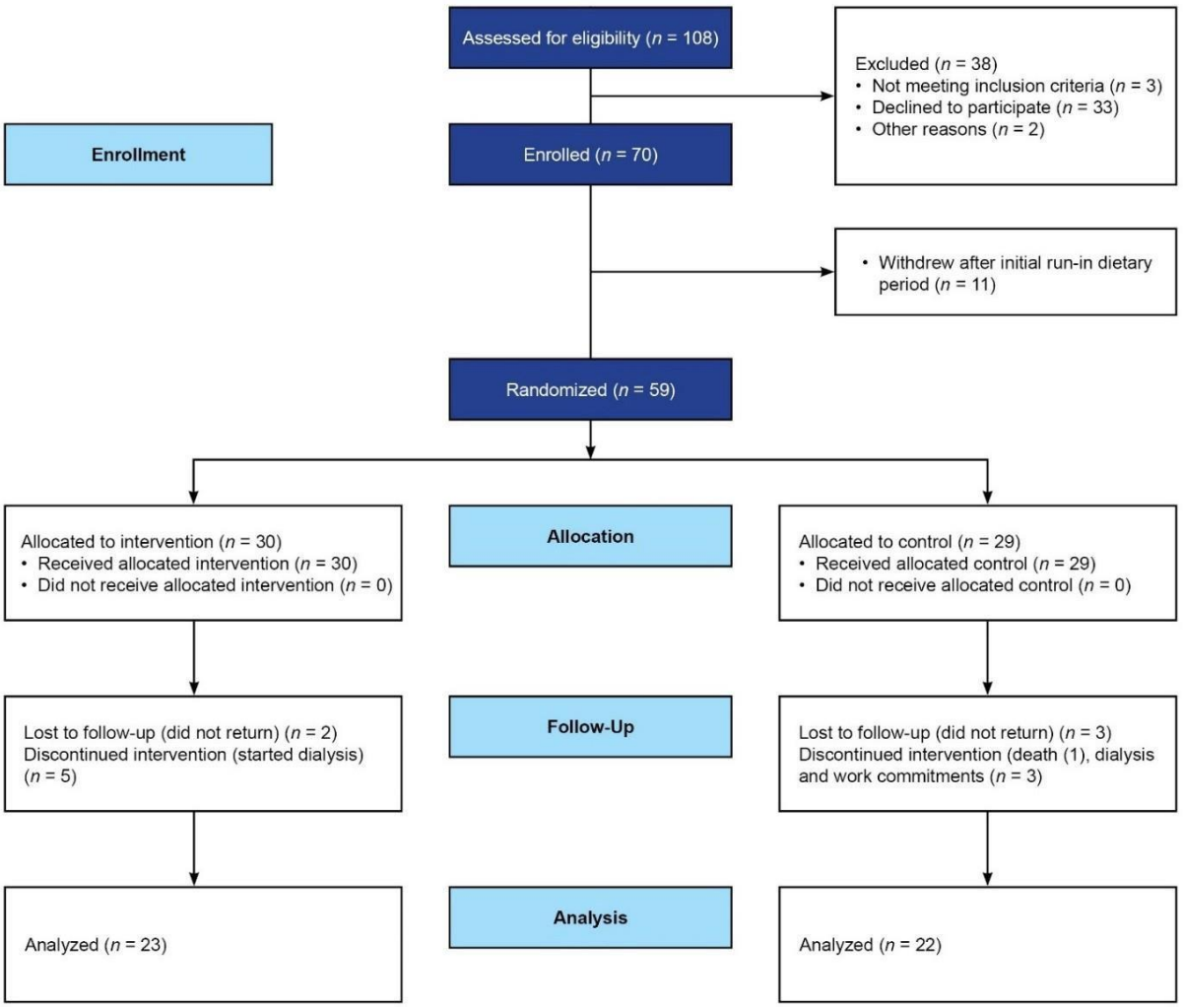

Figure 1. CONSORT flow diagram on the effects of a ß-glucan prebiotic on kidney function, plasma levels of uremic toxins and the gut microbiome in predialysis participants with CKD stage 3 to 5.

2.2. Design

As depicted in Figure 2, all participants had a four-week run-in period on diet only before being randomized to the prebiotic supplement or the diet control group using a simple computer-generated randomized list with an equal allocation ratio. Sequentially numbered sealed opaque envelopes were used to assign group allocation. The principal investigator (PI) was blinded to the treatment. Participants had follow-ups at week 4 (only for issuing product and checking adherence to the diet), week 8 and week 14. They were therefore on the intervention or control for 14 weeks. Nutritional assessments (including anthropometry, biochemical, clinical and dietary intake and adherence scores), blood samples for quantification of uremic toxins and stool samples for characterization of the gut microbiome were obtained at each of the time points, except for week 4. Education of the gut microbiome stool sample collection and completion of the Bristol Stool Scores (BSS) was done at pre-randomization.

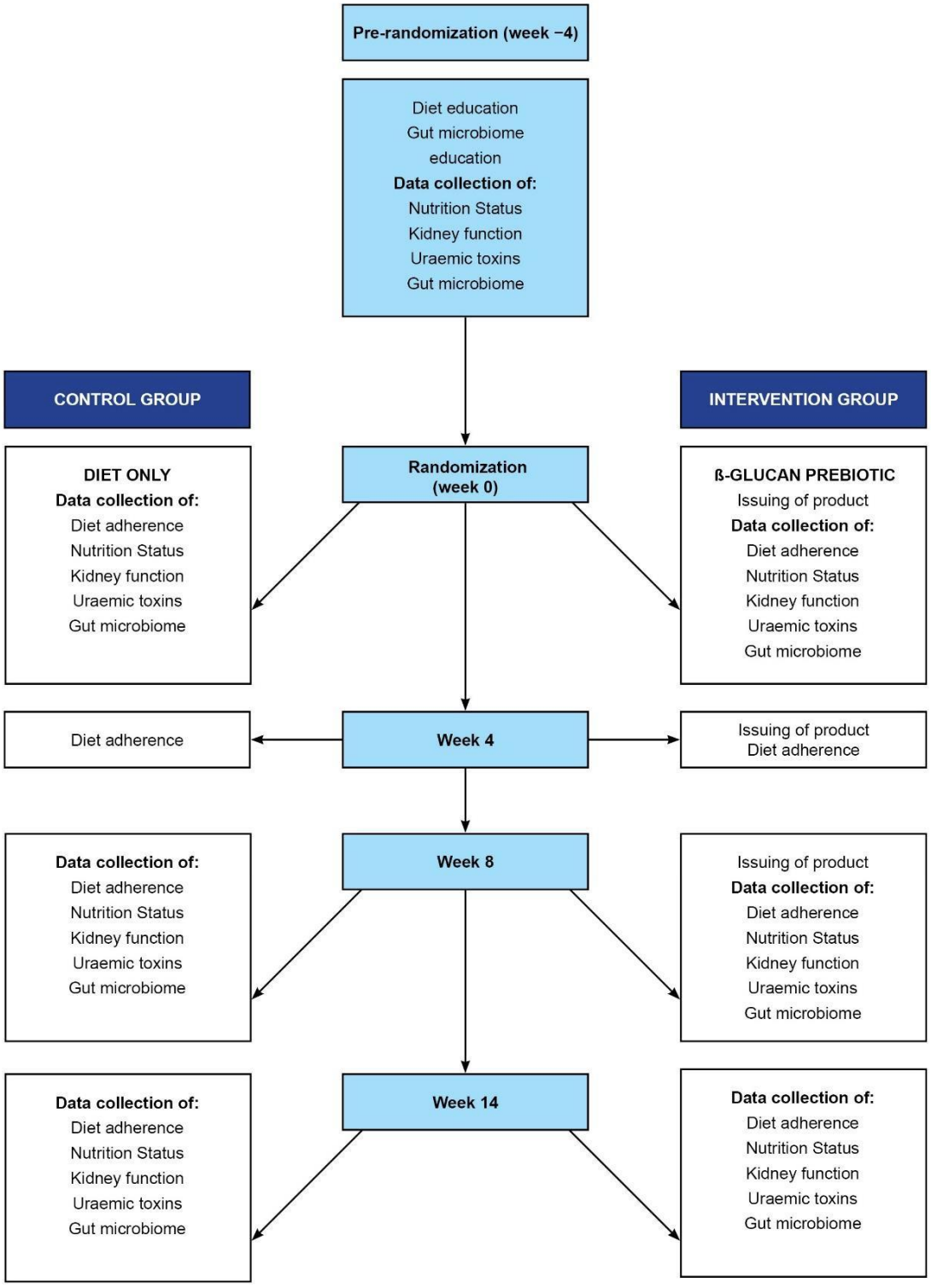

Figure 2. Schematic representation of the study flow; Nutrition status includes anthropometry, clinical, dietary assessment, other nutrition-related biochemical tests.

2.3. Sample Size

Sample size was calculated to estimate a change from baseline to the end of the intervention of 12% in urea, 5% in creatinine and an increase in Lactobacillus of 20% using a two-sample t-test using the Power Analysis and Sample Size software (PASS program), based on a previous study [26]. A 90% power was used. The estimated numbers to detect the change were 16 for urea, 15 for creatinine, and 23 for *Lactobacillus* in each arm of the trial. Therefore, 23 were the minimum required in each group. To compensate for possible withdrawals during the study, 70 participants were enrolled.

2.4. Anthropometry and Biochemistry Methods

The following measurements were performed by the researchers at all three time points: weight, height, waist circumference, and mid-upper arm circumference using standard measures [27]. BMI was calculated, and waist and BMI were interpreted according to WHO standards [28]. Blood was collected by the clinic nurse and sent to the National Health Service laboratory for biochemical analysis. Biochemical tests included kidney function tests, lipid function tests, electrolytes and C-reactive protein (CRP). Blood pressure measurements were performed by the clinic nurse. Participants were asked about gastrointestinal symptoms according to a Likert scale of 'never', 'mild', 'moderate' or 'severe' for the following: nausea, vomiting, flatulence, anorexia, constipation and diarrhea.

2.5. Dietary Intake, Education and Prebiotic Product

The researchers gave in-person dietary advice based on evidence-based CKD guidelines to all patients. The detail of the dietary education has already been described [29]. In short, this entailed education on a simplified diet based on natural, healthy food with the avoidance of take-away, salt and processed foods rich in additives. A protein restriction of 0.8 g/kg was advised. The participants were advised on the diet four weeks before they were randomized to ensure that changes that occurred in the study were not due to dietary changes. The diet was adhered to during the study.

Participants in the IG were advised to use 13.5 g of ß-glucan prebiotic fiber supplement (GlucaChol-22®; manufacturer—At Life Products, Bryanston, South Africa) containing 6 g of fiber of which 3 g is ß-glucan per serving) daily. Dietary tips were given to use the prebiotic supplement in various ways, such as adding it to smoothies, cereals or yoghurts. Participants were given sufficient prebiotic supplement until their next appointment. The PI was responsible for the measurements of participants and assessment of dietary intake. To ensure that the PI remained blinded to the study intervention (the prebiotic supplement), the research assistant was responsible for the issuing of the supplement. Product adherence was measured by the return of empty containers.

Dietary intake was measured at all three time points using an interview-administered 160-item CKD adapted quantified food-frequency questionnaire (FFQ). Portions were calculated to a daily intake and analyzed using the SA foods database [30]. An adapted dietary adherence score sheet was used to measure dietary adherence. There were 12 questions on dietary changes from various food groups with a set of criteria which were selected based on information advised in the infographic and the dietary advice given. The detail of this score sheet and the FFQ have been described [29]. Participants scored a point if they adhered to the criteria, with a maximum of 12 points. An adherence of 10–12 (85– 100%) was classified as excellent adherence, while 8–9 (67–75%) was good adherence, 6–7 (50–58%) was average adherence and <5 (<50%) was considered poor adherence.

2.6. Uremic Toxin Quantification

For the quantification of uremic toxins (UTs), venous blood was collected at all time points (except week 4) in K-EDTA tubes (9 mL). Blood was immediately centrifuged at 2100 x g for 10 min at 4° C. Plasma was aliquoted on ice at 500 µL in sterile tubes, then stored at −80°C before it was sent on dry ice to the Nephrology laboratory of the Ghent University Hospital in Belgium for batch analysis. Total and free concentrations of *p*CS, IxS, *p*CG and IAA were determined by liquid chromatography and fluorescence detection as previously described [31]. In brief, for quantification of the total toxin concentrations, plasma samples were deproteinized by heat, centrifuged and filtered through an Amicon® Ultra 0.5 µL (Merck Millipore Ltd. Carrigtwohill, Ireland) (molecular weight cut-off 30 kDa filter). For the free fraction, the untreated plasma was first filtered through the Amicon filter. The ultrafiltrate was transferred into an autosampler vial, and fluorescein was added as an internal standard. Analysis was performed by ultra-performance liquid chromatography with an Agilent 1290 Infinity device (Agilent, Santa Clara, United States of America). IxS (*λ*ex: 280 nm, *λ*em:376 nm), *p*CS and *p*CG (*λ*ex: 264 nm *λ*em: 290 nm), IAA (*λ*ex: 280 nm, *λ*em: 350 nm), and fluorescein (*λ*ex:443 nm, *λ*em: 512 nm) was detected by an Agilent G1316C fluorescence detector.

2.7. Stool-Sample Collection

All participants were given adequate instructions and stool-sample collection kits together with ice packs and a cooler bag. An information leaflet was also provided. They were asked to collect the sample the day before the study appointment and asked to freeze the sample immediately after collection. After their appointment, the samples were immediately frozen at −80 °C. Participants were also asked to complete the BSS which explains the type and consistency of the stool, ranging from 1 to 7 (from very hard to very loose), the time of the sample collection, and the time of their last stool. The samples were sent on dry ice to VIB laboratories at KU Leuven, Belgium, for further analysis.

2.8. Analysis of Gut Microbiome

To analyze microbiota taxonomic composition, fecal DNA extraction, library preparation and 16S ribosomal RNA (rRNA) gene sequencing were performed as described by Tito et al. [32] using the MiSeq platform at VIB laboratories in Leuven, Belgium.

The gut microbiome results were analyzed using the 16SRNA amplicon method by amplifying the V4 region of the 16S rRNA gene with the 515F and 606R primer (GTGYCAGCMGCCGCGGTAA and GGACTACNVGGGTWTCTAAT, respectively) to produce dual barcoded libraries. This modification was done to contain a barcode sequence between each primer and the Illumina adaptor sequence [33]. Before the sequencing, size selection was performed using Agencourt AMPure (Beckman Coulter, Indianapolis, United States of America) to remove fragments below 200. The Illumina MiSeq plat- form (Illumina, San Diego, United States of America) (MiSeq Reagent Kit v2, 500 cycles,
15.38 % PhiX, 2×250 PE) was used. Fastq sequences were further analyzed per sample using the DADA2 pipeline [34]. This was done after de-multiplexing with sdm, while no mismatching was allowed as part of the LotuS pipeline [35]. After inspecting for quality, sequences were trimmed to remove the primers and the first 10 bases of the primer, which resulted in only 200 bases for R1 files and 130 for R2 files. After removing chimeras and merging sequences, taxonomy was assigned using the formatted RDP training set, 'rdp_train_set_16'.

2.9. Statistical Analysis

Baseline data were described using means and standard deviation for normally distributed continuous variables and medians, and interquartile ranges for non-normally distributed variables. Frequencies were used for categorical data. Data were checked for normality using Kolmogorov tests, histograms and skewness values. To control for proper

randomization, baseline comparisons between the intervention and the control groups were done using t-tests for normally distributed data and Mann–Whitney tests for non-normal data. Categorical data were compared using chi-square tests.

Generalized estimating equation (GEE) models were fitted to identify time effects and differences between the intervention and control group (treatment effects) in terms of kidney function, uremic toxin levels and gut microbiome. In all models, we included the time point (baseline, week 8 and week 14), the treatment group (treatment and control) variables and their interaction as predictors.

A log link with a gamma distribution for the residual was chosen to model severely non-normal variables, including biochemistry, uremic toxin levels and dietary data. An identity link with the normal distribution of residuals was used to model anthropometric data. The models were fitted by using a Huber–White robust estimator with an unstructured correlation matrix.

All analyses were conducted using IBM®SPSS® version 27 (10 November 2021). A 5% level of significance was used to reject the null hypothesis in statistical tests (two sides). A p-value of < 0.05 was considered significant.

2.10. Gut Microbiome Statistical Analysis

Statistical analyses were performed using R Statistical Software version 3.6.1 (http://www.r-project.org/, 15th September 2021).

Prior to analysis, 16S reads were rarefied to an even depth of 10,000 reads per sample; genera with low prevalence (present in less than 20% of the samples) were excluded from further tests. Alpha diversity, using the Shannon Diversity Index, was calculated using an in-house script. The Principal Coordinate Analysis and other analyses based on the Bray–Curtis distances were done using Scikit-learn (v0.24.1) and Scikit-bio (v0.5.5) using Python (v3.8.2) [36,37]. Comparisons between groups were made using the package statannot, which applies a pairwise Kruskal–Wallis test with Bonferroni correction for multiple testing. Genus-level, microbiome profiles variation explained by various features in the metadata was performed using univariate or multivariate stepwise distance-based redundancy analysis as implemented in R (v3.6.1) using the rda function from the vegan (v2.5-6) package [38]. Group-wise comparisons at genus level were performed using ALDEx2 (v1.18.0, ran using R v3.6.1), with mc.samples = 256, leaving other parameters at their default values [39]. The function T-test was used with default parameters (either in paired or unpaired mode, depending on the comparison). Correlations between genera and metadata were established using FlashWeave (v0.18 ran with Julia 1.6.1), using default parameters [40].

Samples' community types, hereafter referred to as enterotypes, were obtained by combining 16S rRNA gene data from this cohort with data from the Flemish Gut Flora Project (FGFP) [35] and applying an approach based on Dirichlet Multinomial Mixtures (DMM) [36].

3. Results

3.1. Baseline Data

Fifty-nine participants were enrolled in the trial from August 2018 to December 2019; 30 were randomized to the intervention group and 29 to the control group (Figure 1). Baseline characteristics are described in Table 1. Participants had a mean age of 41.0 ± 11.9 years and were predominantly female. The main cause of kidney failure as documented in the medical files was hypertension. Half of the participants were unemployed, with most participants having a monthly income of less than US $126.

The overall BMI was 28.3 ± 6.6 kg/m^2, with a high prevalence of overweight and obesity in 67.7% of participants. Biochemistry and the plasma levels of uremic toxins are shown in Table 2 and are reflective of CKD.

The baseline protein intake percentage was 15% of energy intake, which was 0.7 g

per kg of ideal body weight for the group at baseline, carbohydrate intake was 56% and fat intake 33% of energy intake, of which saturated fat intake was 8.6%. Fiber intake was 17.6 g (14.1, 21.3), while total sugar intake was 61.5 ± 23 g. Mineral intake is shown in Table 1.

There were no significant differences between the intervention and the control group in baseline characteristics except for slightly higher high-density (HDL) levels in the intervention group.

Table 1. Baseline characteristics of participants at randomization.

		Total (*N* = 59)	Intervention Group (*N* = 30)	Control Group (*N* = 29)	*p* Value
Age (years) (mean)		41.0 ± 11.6	40.6 ± 11.4	41.3 ± 12.0	0.082
Socio-demographics		***N* (%)**	***N* (%)**	***N* (%)**	
Sex	Male	25 (42.4)	11 (44)	14(56)	0.367
	Female	34 (57.6)	19 (55.6)	15 (44.1)	
Income (/month)	$0–$126	24 (40.7)	10 (41.6)	14 (58.3)	0.332
	$127–$316	16 (27.1)	7 (43.8)	9 (56.2)	
	$317–$633	13 (22.0)	8 (61.5)	5 (38.4)	
	$634–$949	4 (6.8)	3 (75.0)	1 (25.0)	
	>$949	2 (3.4)	2 (100.0)	0	
Employment	Employed	29 (49.2)	17 (58.6)	12 (41.3)	0.240
	Unemployed	30 (50.8)	13 (43.3)	17 (56.6)	
Clinical data		***N* (%)**	***N* (%)**	***N* (%)**	
Cause of kidney failure	Polycystic kidneys	3 (5.1)	1 (33.3)	2 (66.6)	0.456
	Hypertension	29 (49.2)	13 (44.8)	16 (55.2)	
	Glomerular disease	13 (22.0)	9 (69.2)	4 (30.7)	
	Other	14 (23.7)	7 (50.0)	7 (50.0)	
GFR categories (mL/min/1.73 m²)	30–59 Stage 3	19 (32.2)	9 (47.3)	10 (52.6)	0.867
	15–29 Stage 4	16 (27.1)	9 (56.2)	7 (43.8)	
	<15 Stage 5	24 (40.7)	12 (50.0)	12 (50.0)	
		Median Interquartile range (IQR)	**Median (IQR)**	**Median (IQR)**	
Blood pressure	**Systolic (mmHg)**	142.0 (128.3, 168,3)	145.0 (134.0, 170.0)	140.0 (136.0, 147.0)	0.549
	Diastolic (mmHg)	80.0 (72.0, 92.8)	82.0 (72.0, 93.0)	80.0 (78.0, 91.0)	0.765
Anthropometry		**Mean ± standard deviation (SD)**	**Mean ± SD**	**Mean ± SD**	
Weight (kg)		75.7 ± 20.7	73.5 ± 18.**5**	78 ± 22.9	0.461
BMI (kg/m²)		28.3 ± 6.6	27.4 ± 6.2	29.2 ± 6.99	0.300
Waist circumference (cm)		89.6 ± 15.0	86.9 ± 13.1	92.4 ± 16.5	0.161
MUAC (cm)		30.2 ± 5.1	29.8 ± 5.2	30.7 ± 5.1	0.527
		***N* (%)**	***N* (%)**	***N* (%)**	
BMI categories	Underweight	3 (5.0)	2 (6.7)	1 (3.4)	0.791
	Normal weight	16 (27.1)	10 (62.5)	6 (37.5)	
	Overweight	19 (32.2)	8 (42.1)	11 (57.9)	
	Obese	21 (35.5)	10 (47.6)	11 (52.3)	

Abbreviations: GFR: glomerular filtration rate, IQR: interquartile range, SD: standard deviation, BMI: body mass index, MUAC: mid-upper arm circumference.

Table 2. Baseline biochemistry and uremic toxin levels of participants at randomization.

	Total (*N* = 59)	**Intervention Group (*N* = 30)**	**Control Group (*N* = 29)**	***p* Value**
Biochemistry	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	
Urea (mmol/L)	14.2 (9.1, 28.6)	14.4 (11.5, 21.0)	14.1 (10.9, 28.1),	0.952
Creatinine (mmol/L)	232.0 (175.0, 461.0)	230.0 (208.0, 53.0)	308.0 (187.0, 33.0)	0.921
GFR (mL/min.1.73) m²	20.0 (11.0, 35.0)	21.0 (13.0, 27.0)	19.0 (11.0, 35.0)	0.976
Potassium (mmol/L)	5.0 ± 0.7	4.9 ± 0.8	5.0 ± 0.7	0.509
Phosphate (mmol/L)	1.16 (1.0, 1.5)	1.2 (1.1, 1.4)	1.1 (1.0, 1.5)	0.844
Cholesterol (mmol/L)	4.7 ± 1.1	4.7 ± 1.1	4.8 ± 1.2	0.958
LDL (mmol/L)	2.6 ± 1.0	2.6 ± 0.8	2.6 ± 1.1	0.859
HDL (mmol/L)	1.1 (0.9, 1.3)	1.2 (1.1, 1.4)	1.0 (0.9, 1.1)	**0.032**
Triglycerides (mmol/L)	1.6 (1.2, 2.4)	1.4 (1.2, 1.9)	1.9 (1.4, 2.6)	0.079
CRP (mg/L)	4.0 (2.0, 8.0)	5.0 (2.0, 8.0)	3.0 (2.0, 5.0)	0.760
Uremic toxins	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	
Total IxS (mg/L)	3.96 (1.55, 10.27)	3.44 (2.01, 4.93)	5.28 (2.84, 8.32)	0.533
Free IxS (mg/L)	0.10 (0.03, 0.26)	0.06 (0.06, 0.14)	0.13 (0.64, 0.22)	0.724
Total *p*CS (mg/L)	5.69 (2.86, 10.37)	5.08 (4.20, 7.12)	5.81 (4.09, 7.24)	0.840
Free *p*CS (mg/L)	0.14 (0.06, 0.30)	0.14 (0.09, 0.28)	0.14 (0.67, 0.19)	0.391
Total *p*CG (mg/L)	0.10 (0.03, 0.26)	0.11 (0.03, 0.21)	0.10 (0.04, 0.17)	0.920
Free *p*CG (mg/L)	0.09 (0.02, 0.20)	0.10 (0.03, 0.18)	0.09 (0.03, 0.15)	0.933
Total IAA (mg/L)	0.80 (0.49, 1.33)	0.66 (0.55, 1.12)	0.92 (0.70, 1.06)	0.662
Free IAA (mg/L)	0.12 (0.09, 0.25)	0.11 (0.10, 0.24)	0.14 (0.11, 0.23)	0.906
Dietary intake	**Median (IQR) Mean ± SD**	**Median (IQR Mean ± SD**	**Median (IQR) Mean ± SD**	
Energy (kcal)	5710 (4480.0, 6982.0)	5685.6 (5149.9, 6662.1)	5955.52 (4528.0, 6704.8)	0.773
Protein (g)	51.9 ± 20.5	54.3 ± 17.0	49.5 ± 23.7	0.085
Plant protein (g)	16.6 (14.0, 21.1)	16.7 (15.1, 19.5)	15.6 (14.5,20.7)	0.544
Animal protein (g)	32.5 ± 14.5	34.5 ± 12.0	30.3 ± 16.6	0.127
Total sugar (g)	61.5 ± 23	60.3 ± 21.5	62.6 ± 24.7	0.298
Fiber (g)	17.6 (14.1, 21.3)	17.6 (16.9, 19.9)	16.2 (14.1, 20.5)	0.448
Fat intake (g)	50 (35.1, 60.8)	47.7 (40.5, 53.7)	50.3 (38.4, 55.4)	0.785
Saturated fat (g)	13.05 (9.6,18.8)	13.1 (11.7, 17.0)	13.1 (9.4, 15.4)	0.371
Trans fat (g)	0.3 (0.1,0.6)	0.3 (0.2, 0.5)	0.3 (0.2, 0.6)	0.844
Potassium (mg)	1923.0 (1553.5, 2405.4)	2048.9 (1751.9, 2338.6)	1774.5 (1564.4, 2267.7)	0.396
Phosphate (mg)	735.6 (523.0, 939.7)	767.8 (638.6, 865.3)	581.3 (521.4, 848.4)	0.102
Sodium (mg)	1829.23 (1290.4, 2584.8)	1999.3 (1666.6, 2435.5)	1803.8 (1186.5, 2090.0)	0.907

Abbreviations: GFR: glomerular filtration rate, LDL: low-density lipoprotein, HDL: high-density lipoprotein, CRP: C-reactive protein, IxS: Indoxyl sulfate, *p*CS: *p*-cresyl sulfate, *p*CG: *p*-cresyl glucuronide, IAA: indole-3-acetic acid. Bold if $p < 0.005$.

3.2. Post-Intervention Data

Forty-five participants completed both arms of the trial in full. (Figure 1).

3.3. Kidney Function and Biochemistry

There were no significant changes in serum urea and creatinine concentrations or glomerular filtration rate over 14 weeks (Table 3). There was a significant reduction in low-density lipoprotein (LDL) cholesterol in the intervention group (Table 4), with a significant treatment effect. At week 8, the decrease in the intervention group was 0.88 timesthe decrease in the control group, but at week 14 the difference disappeared. Total

cholesterol, HDL cholesterol and triglycerides (TG) did not change (Table 4). All the other biochemical values, including potassium, phosphate, sodium, and CRP, remained unchanged throughout the study (Supplementary Table S1). CRP was raised (>3 mg/L) in 64% of participants.

Table 3. Generalized estimation equation models for change in kidney function over time in the intervention over the control group (proportions).

	Model 1 Outcome: Urea			**Model 2 Outcome: Creatinine**			**Model 3 Outcome: eGFR**		
Parameter	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI ***	***p***
[Intervention group]	0.93	0.66–1.30	0.669	0.92	0.63–0.35	0.625	0.97	0.66–1.43	0.869
[week 8]	1.05	0.92–1.19	0.479	1.02	0.94–1.10	0.613	1.02	0.93–1.12	0.710
[week 14]	1.01	0.88–1.16	0.850	1.01	0.89–1.14	0.891	1.02	0.93–1.12	0.636
[Intervention group] *[week 8]	1.14	0.98–1.33	0.092	1.12	0.97–1.28	0.118	0.95	0.84–1.06	0.244
[Intervention group] *[week 14]	1.06	0.90–1.25	0.514	1.03	0.87–1.22	0.718	0.93	0.81–1.05	0.340

Exp: exponential; b = estimated model coefficient; *p* = *p*-value (Wald χ^2 test); eGFR: estimated glomerular filtration rate; *Intervention by time treatment effect.

Table 4. Generalized estimation equation models for change in lipid values over time in the intervention over the control group (proportions).

	Model 4: Outcome: Total Cholesterol			**Model 5 Outcome: LDL Cholesterol**			**Model 6 Outcome: HDL**			**Model 7 Outcome: TG**		
Parameter	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI**	***P***	**Exp (b)**	**95% CI**	***p***
[Intervention group]	0.98	0.88–1.12	0.997	1.01	0.84–1.24	0.856	1.25	1.02–1.54	**0.031**	0.69	0.50–0.95	0.694
[week 8]	1.02	0.96–1.08	0.476	1.07	0.97–1.17	0.170	1.07	1.02–1.13	**0.010**	0.86	0.75–1.00	0.057
[week 14]	0.98	0.91–1.05	0.594	1.00	0.88–1.14	0.971	1.01	0.91–1.11	0.866	0.90	0.74–1.10	0.307
[Intervention group] * [week 8]	0.93	0.86–1.01	0.571	0.88	0.76–0.99	**0.037**	0.97	0.90–1.05	0.486	1.03	0.84–1.26	0.777
[Intervention group] * [week 14]	1.03	0.93–1.15	0.105	1.02	0.86–1.22	0.776	1.03	0.91–1.15	0.661	1.02	0.81–1283	0.858

Exp: exponential; b = estimated model coefficient; *p* = *p*-value (Wald χ^2 test); LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; TG: triglycerides, Bold if $p < 0.05$; * Intervention by time treatment effect.

3.4. Plasma Levels of Colon-derived Uremic Toxins

There was a significant decrease of free IxS over time in the intervention group, with a significant treatment effect (Table 5). The decrease in the intervention group was 0.46 times the decrease in the control group during week 8 ($p = 0.003$), and 0.35 times during week 14 ($p < 0.001$). Supplementary Figures S1–S3 graphically show the mean change over time in uremic toxins.

There was a significant intervention effect on free *p*CS; the decrease in the intervention groups was 0.48 times the decrease in the control group at week 14 ($p = 0.006$).

There was a significant decrease in total *p*CG and free *p*CG at week 14, with a significant intervention effect. For total *p*CG, the decrease in the intervention group was 0.14 times the decrease than in the control group ($p < 0.001$) at week 14, and for free *p*CG, the decrease in the intervention group was 0.13 times the decrease than in the control group ($p < 0.001$) at week 14. Free IAA was 0.56 times the decrease in the intervention vs. the control group at week 14, with it being very close to statistical significance ($p = 0.051$).

Table 5. Generalized expected equations model for changes in plasma levels of uremic toxins over time (mg/L) in the intervention over the control group (proportions).

	Model 1: Outcome: Total IxS			**Model 2: Outcome: Free IxS**			**Model 3: Outcome: Total *p*CS**			**Model 4 Outcome: Free *p*CS**			**Model 5 Outcome: Total *p*CG**			**Model 6 Outcome: Free *p*CG**			**Model 7 Outcome: Total IAA**			**Model 8 Outcome: Free IAA**		
Parameter	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI**	***p***	**Exp (b)**	**95% CI**	***p***
[Intervention group]	0.88	0.47–1.62	0.674	1.78	0.65–0.09	0.264	1.16	0.76–1.78	0.479	1.61	0.77–3.40	0.208	3.40	0.86–13.5	0.081	0.34	0.84–1.44	0.087	1.06	0.65–1.74	0.798	1.72	0.77–0.86	0.185
[week 8]	1.08	0.95–1.24	0.211	1.14	0.93–1.40	0.211	0.87	0.83–1.20	0.968	1.06	0.82–1.38	0.630	0.77	0.35–1.87	0.508	0.35	0.12–0.98	**0.045**	1.13	1.00–1.28	0.050	1.13	0.96–1.33	0.148
[week 14]	1.09	0.96–1.21	0.227	1.06	0.84–1.33	0.591	1.00	0.68–1.11	0.258	0.83	0.61–1.12	0.277	1.14	0.77–1.69	0.530	0.16	0.09–0.29	**<0.001**	1.04	0.86–1.26	0.655	1.03	0.84–1.27	0.749
[Intervention group]*[week 8]	0.97	0.71–1.31	0.863	0.46	0.28–0.76	**0.003**	1.09	0.85–1.36	0.952	0.96	0.72–1.28	0.772	0.51	0.15–1.75	0.286	0.45	0.12–1.69	0.241	1.08	0.86–1.35	0.515	0.83	0.50–1.36	0.449
[Intervention group]*[week 14]	0.93	0.68–1.26	0.622	0.35	0.21–0.60	**<0.001**	0.99	0.74–1.32	0.547	0.48	0.29–0.82	**0.006**	0.14	0.08–0.28	**<0.001**	0.13	0.06–0.27	**<0.001**	1.03	0.81–1.31	0.797	0.56	0.31–1.00	0.051

Exp: exponential; b: estimated model coefficient; *p* = *p*-value (Wald χ^2 test); IxS: Indoxyl sulfate, *p*CS: *p*-cresyl sulfate, *p*CG: *p*-cresyl glucuronide, IAA: indole-3-acetic acid; Bold if $p < 0.05$; *Intervention by time treatment effect

3.5. Anthropometry, Dietary Changes and Adherence to the Diet

There were no significant anthropometrical, and dietary changes from baseline to the end of the study (Supplementary data—Tables S2 and S3).

Diet adherence was excellent (above 85% throughout) and improved as the study progressed (Supplementary data—Table S4).

Compliance with the product as measured by the return of empty containers was good, with an 89.6% compliance at week 4, a 90.4% compliance at week 8, and an 82.7% compliance at the end of the study. Some participants did not return their containers, although they reported that they were using the prebiotic. Nearly all participants (>90%) did not experience gastrointestinal symptoms such as nausea, vomiting, flatulence, anorexia, constipation and diarrhea at baseline, and this did not change over time between the twogroups.

3.6. Gut Microbiome

The most abundant genera were *Faecalibacterium*, *Prevotella*, *Bacteroides*, *Blautia* and *Roseburia* as shown in Figure 3. Although there was a trend to increase in *Prevotella* and a trend to reduction in *Bacteroides* and *Blautia* in the intervention group, this was not significant after correcting for multiple testing using ALDEx2. (Supplementary data—Table S5).

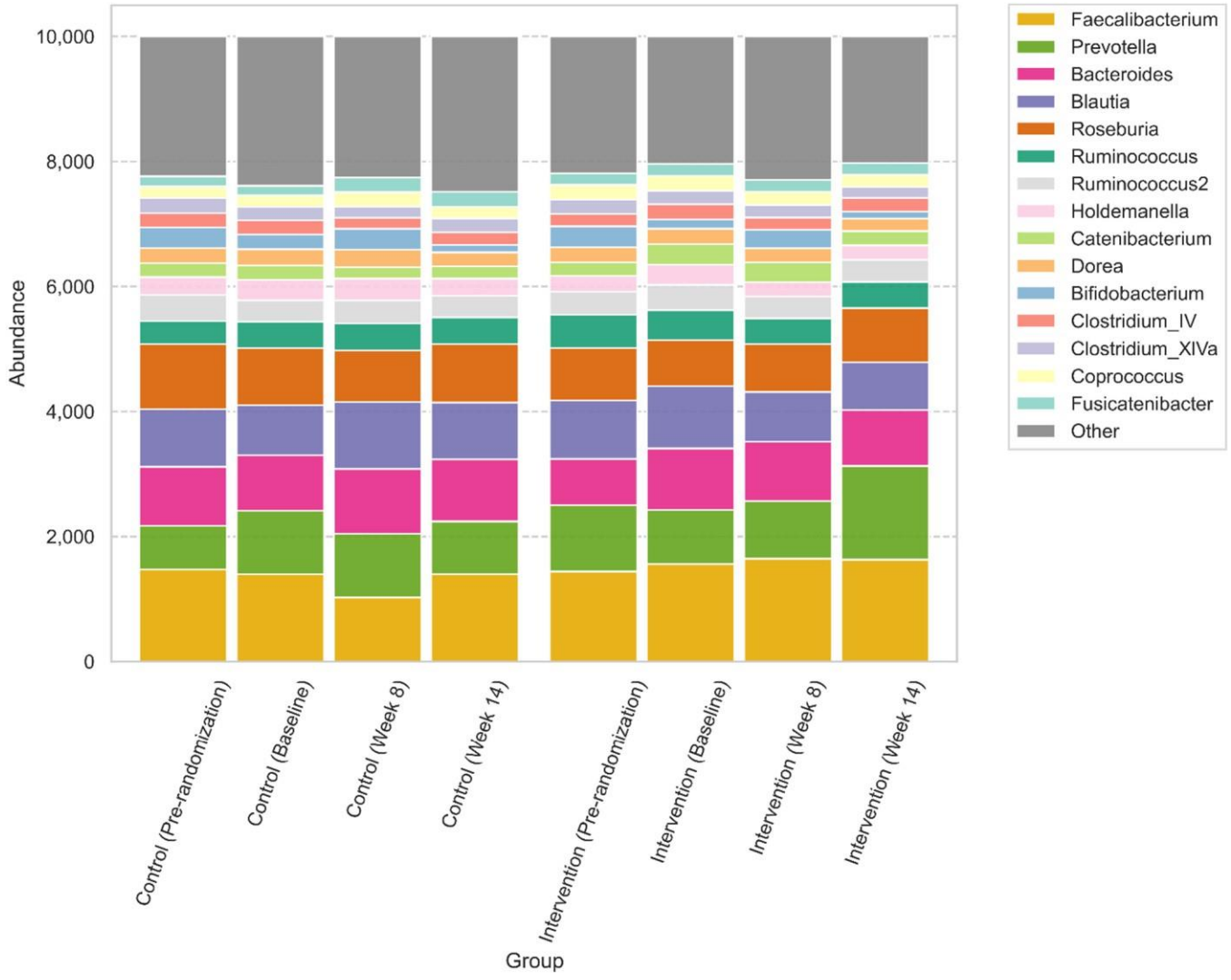

Figure 3. Relative abundances at the genera level.

Gut microbiota were characterized by a high relative abundance of *Prevotella* and *Bacteroides* 2 as shown in Figure 3. This was further confirmed by enterotyping using the Flemish Gut Flora Project as a background (Supplemental Figure S4), where all participants were assigned to either the *Prevotella* or *Bacteroides* 2 enterotype [41]. There were no participants with the *Ruminococcus* or *Bacteroides* 1 enterotype present in this cohort. There were no significant differences in relative abundances between groups using ALDEx2 (Supplementary data—Table S5).

When comparing the intervention and the control group as shown in Figure 4, the relative abundance of *Bacteroides* 2 was slightly higher in the intervention group at randomization; however, this lowered over the study period from 52% to 45%, whereas it went up in the control group from 50 to 60%. However, these within-group differences were not statistically significant (ALDEx2 test, $p = 0.298$).

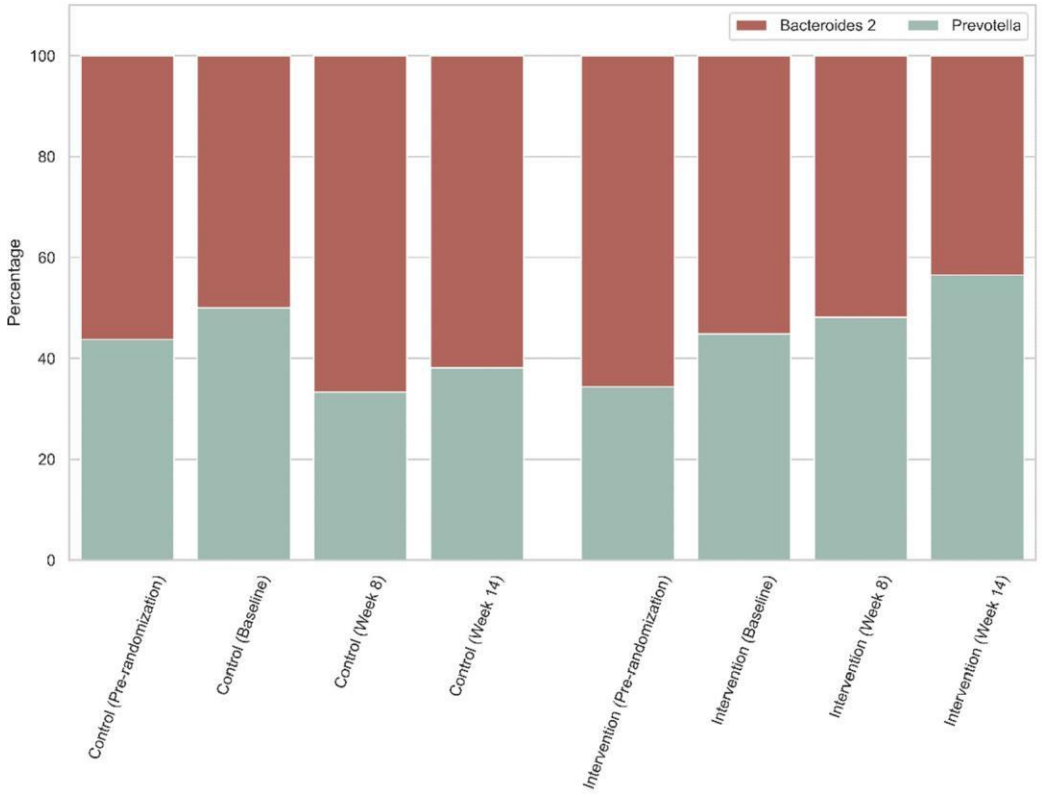

Figure 4. Enterotype percentages of the intervention and control group over time.

Figure 5A shows the principal coordinate analysis of inter-individual differences (beta diversity) by Bray–Curtis dissimilarity. The inter-individual Bray–Curtis distance was significantly higher in the control group than the intervention group at baseline ($p < 0.0001$) and remained higher throughout the total study period. Furthermore, a significant difference was observed between the baseline and week 8 in the control group ($p < 0.001$) (Figure 5B). There were no differences in alpha diversity between the intervention and control group (Shannon index) at randomization and week 8 or 14 (Figure 5C).

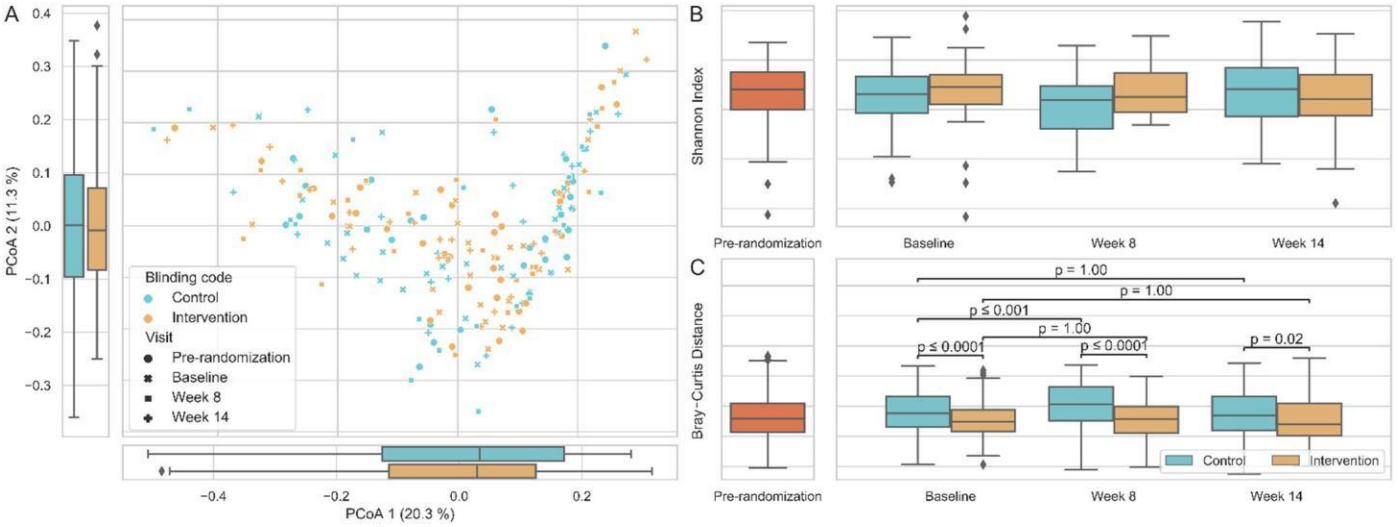

Figure 5. Alpha and beta diversity comparisons by groups. (**A**) Principal coordinate analysis (PCoA2) of inter-individual differences (beta diversity) by Bray–Curtis dissimilarity. (**B**) Alpha diversity according to the Shannon index. (**C**) Within-group inter-individual Bray–Curtis distance.

The redundancy table analysis (RDA) (Figure 6) shows that the following non-redundant factors influenced the gut microbiome's compositional variation by 12.1%: triglyceride levels (step stepwise dbRDA, $R^2 = 2.97\%$, $p < 0.001$), the cause of kidney failure (stepwise dbRDA, $R^2 = 1.96\%$, $p < 0.001$), gender (stepwise dbRDA, $R^2 = 1.56\%$, $p < 0.001$, BMI (stepwise dbRDA, $R^2 = 0.82\%$, $p = 0.002$), GFR (stepwise dbRDA, $R^2 = 0.73\%$, $p = 0.002$), age (stepwise dbRDA, $R^2 = 0.60\%$, $p = 0.002$), the prebiotic intervention (stepwise dbRDA, $R^2 = 0.55\%$, $p = 0.002$), high-density lipoprotein (HDL) (stepwise dbRDA, $R^2 = 0.69\%$, $p < 0.001$), low-density lipoprotein (LDL) (stepwise dbRDA, $R^2 = 0.46\%$, $p = 0.008$), free IAA (stepwise dbRDA, $R^2 = 0.39\%$, $p = 0.016$), total sugar intake (stepwise dbRDA, $R^2 = 0.45\%$, $p = 0.008$), urea (stepwise dbRDA, $R^2 = 0.34\%$, $p = 0.019$), free *p*CS (stepwise dbRDA, $R^2 = 0.30\%$, $p = 0.036$), and finally alcohol intake (stepwise dbRDA, $R^2 = 0.29\%$, $p = 0.049$).

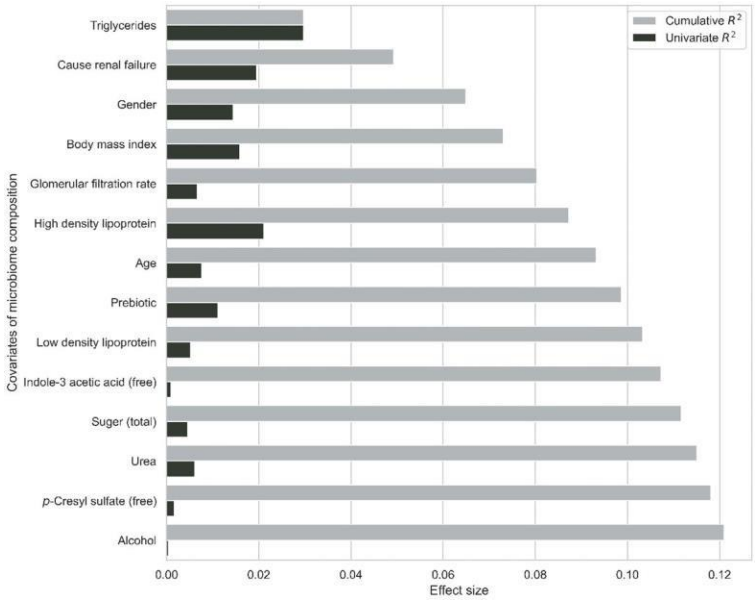

Figure 6. Cumulative effect size of covariates affecting the compositional variation of the gut microbiome selected by the redundancy analysis (grey bars) compared with individual effect sizes assuming independence (black bars).

From Table 6, it can be seen that many genera were positively and negatively correlated with the various uremic toxins and other outcomes. *Bifidobacterium* correlated negatively with urea, while *Gemmiger* correlated negatively with creatinine. *Blautia, Clostridium_XlVa, Acetanaerobacterium* correlated positively with TG, while *Prevotella* correlated positively with HDL. *Bulleidia* correlated negatively with total IxS, while *Ruminococcus2* correlated positively. Weight was positively correlated to *Dialister* and *Butyricicoccus*, while BMI was negatively correlated to *Howardella*. *Faecalibacterium* correlated negatively with total *p*CS, while correlating positively with *Methanobrevibacter*, *Desulfovibrio* and *Peptococcus*. Free *p*CS correlated positively with *Escherichia/Shigella.*

Table 6. Correlations of genera to biochemistry and uremic toxins.

Variable	Genus	* Correlation Value
Urea	*Bifidobacterium*	−0.266
Creatinine	*Gemmiger*	−0.177
LDL cholesterol	*Mogibacterium*	−0.218
HDL cholesterol	*Prevotella*	0.202
TG	*Blautia*	0.197
	Clostridium_XlVa	0.205
	Acetanaerobacterium	0.180
Weight	*Dialister*	0.236
	Butyricicoccus	0.234
BMI	*Howardella*	−0.182
Total IxS	*Ruminococcus2*	0.211
	Bulleidia	−0.235
Total *p*CS	*Faecalibacterium*	−0.216
	Methanobrevibacter	0.288
	Desulfovibrio	0.183
	Peptococcus	0.259
Free *p*CS	*Escherichia/Shigella*	0.222
Total IAA	*Mogibacterium*	0.330
	Howardella	0.301

Abbreviations: TG: triglycerides, LDL: low-density lipoprotein, HDL: high-density lipoprotein, IxS: Indoxyl sulfate, *p*CS: *p*-cresyl sulfate, *p*CG *p*-cresyl glucuronide, IAA: indole-3-acetic acid * significant correlations.

4. Discussion

In this intervention study, the supplementation of the prebiotic β-glucan resulted in a decrease in especially the free concentrations of the colon-derived uremic toxic levels IxS, *p*CS and *p*CG, without a change in kidney function over the 14-week study period. There was a trend to shift from a *Bacteroides* 2 to *Prevotella* enterotype (going from 50% of subjects having the *Prevotella* enterotype at baseline to 38% at week 14 in the control group, opposed to 45% going to 57% in the intervention group, Figure 4), and as there were no changes in dietary intake, these changes may be ascribed to the prebiotic intervention. The RDA analysis showed that the prebiotic significantly affected the gut microbiome. As *Bacteroides* 2 is associated with various conditions, especially inflammation, the trend to shift from *Bacteroides* 2 to *Prevotella* may be considered beneficial.

Previous studies show varying results of prebiotics on kidney function [42]. The effect of prebiotics on kidney function is small [22], in agreement with a recent meta-analysis which showed a reduction in urea, but not creatinine [43]. These changes were not influenced by the dose of fiber, the intervention time or dialysis [43]; the reduction in urea may be due to the fiber intake promoting the growth of colonic bacteria to incorporate nitrogen,

resulting in an increased fecal excretion. However, in the present study, no significant differences in the relative abundances of urease-producing genera were observed between groups. In addition, urea may have remained stable owing to the participants' low protein intake.

Patients with CKD have a high risk of cardiovascular mortality [5]. Dyslipidemia is one of the many traditional risk factors contributing to the development of CVD in the CKD population [44]. In addition, elevated uremic toxins levels have been associated with cardiovascular damage [45]. Dyslipidemia in the later stages of CKD is characterized by increasing total cholesterol and LDL as GFR decreases [46]. In the current study, there was a significant change in LDL cholesterol after 8 weeks, but not at 14 weeks. This reduction may be due to the effect of the ß-glucan prebiotic, since there were no significant changes in dietary intake over time. The ß-glucan prebiotic has been shown to reduce LDL cholesterol in 'at risk' CVD patients [23], which is similar to the present findings. Potential mechanisms through which gut microbiota may affect circulating lipid levels could be through the gel-forming property of ß-glucan reducing cholesterol and bile salt absorption, the action of bile salt hydrolase (BSH) [23], as well as through the action of SCFAs [47].

There was a significant reduction in especially the free plasma levels of the colon-derived uremic toxin levels IxS, *p*CS and *p*CG, which might be beneficial since free levels of specific uremic toxins are associated with overall mortality and CVD risk [48]. Additionally, free *p*CS and free IxS were also significantly associated with the gut microbiome according to the RDA analysis. The effect of prebiotics on uremic toxin levels in CKD patients varies; some studies show changes in *p*CS but not IxS. *p*CS and *p*CG are the main conjugates of *p*-cresol, which is generated as an end product of tyrosine metabolism and contributes to uremic toxicity [49]. The reduction in *p*CG is a positive and novel finding and may be linked to the prebiotic fiber intake in the intervention group. Advanced CKD has been linked to increased glucuronidation of *p*-cresol and to CVD and mortality [50]. If fiber is sufficient, gut bacteria use less protein as an energy source, resulting in lower uremic toxin production [48]. Studies have shown significant reductions in *p*CS [51] and IxS [24] with prebiotic use in hemodialysis patients, while synbiotics showed significant changes in *p*CS in predialysis patients [52] and hemodialysis patients [53], with one study showing a reduction in IxS in hemodialysis patients [54]. Wu et al. [55] reported only on a reduction of *p*CS in their meta-analysis of fiber supplementation effects on uremic toxins, while in a very recent meta-analysis, Yang et al. [43] showed that fiber supplementation reduced *p*CS and IxS significantly. They found that the reduction in *p*CS was not influenced by dose, dialysis, diabetes or intervention time, while a significant reduction in IxS was found in dialysis patients. Another possible reason for these positive changes that requires further exploration is that SCFA production alters intestinal integrity, thereby preventing entry of uremic toxins into blood circulation [43].

Gryp et al. [16] reported that the bacterial species involved in phenolic compound generation were mostly from the *Bacteroidetes* and *Firmicutes* phyla and suggested to target *Bacteroides* in interventions. While studies have shown *Prevotella* to be negatively associated with *p*CS and IxS [56], it may be that the trend to shift from *Bacteroides* 2 to *Prevotella* enterotype in the intervention group, contributed to the reduction of these uremic toxins. Furthermore, *Desulfovibrio*, *Methanobrevibacter*, and *Peptococcus* were positively correlated with *p*CS. The abundance of *Desulfovibrio* has been associated with a lower GFR [57]. *Faecalibacterium* was negatively correlated with *p*CS in the current study; it has been found to be lower in CKD patients and has been found to have a positive correlation with GFR [17,58]. *Methanobrevibacter* has been reported to be pro-inflammatory, while *Peptococcus* is considered to be a pathogen [59,60]. The correlations of the uremic toxins were associated with genera that are reflective of CKD dysbiosis and inflammatory conditions.

The total sugar intake, although within recommended guidelines, was found to significantly affect the gut microbiome according to the RDA analysis. Stanford et al. have linked microbiome variation to individual food groups with a high sugar content [56]. A high sugar intake modifies the ratio of *Bacteroidetes* and *Proteobacteria*, to have increased

pro-inflammatory properties, and reduced immune functioning [61]. The baseline dietary intake was quite varied in this current study. This pattern may have also contributed to the higher *Bacteroides* 2 prevalence than *Prevotella* at baseline in the group overall.

While the α-diversity was not significantly different, there were differences in the ß-diversity. The higher ß-diversity in the control group may be linked to the lower trend in animal protein intake, studies have shown that microbial diversity is lower in diets high in animal protein [62]. Increased dietary fiber has been linked to a reduction in gut inflammation, which in turn is often associated with low bacterial loads and diversity, resulting in the *Bacteroides* 2 enterotype [19]. Furthermore, fiber has been found to increase *Prevotella* genera [63], which may explain some of the trends seen, owing to the addition of the prebiotic fibre. Prebiotic fiber enhances the selective stimulation of indigenous bacteria in the gut that results in many benefits to the host [62]. This trend in the shift away from the *Bacteroides* 2 enterotype in the intervention group is in line with observations in other studies [20,21]. Though given transitions from one enterotype to another are rare (Vandeputte D et al. unpublished data), a larger cohort would be required to find significant results here. Studies on the gut microbiome in CKD patients show changes in *Bifidobacterium* and *Lactobacillus* with synbiotics and lactulose [52,64]; however, there were no significant changes in the abundances of these genera in this current study. It may be that these abundances were relatively low to start with. CKD has been associated with lower intestinal colonization of *Bifidobacterium* and *Lactobacillus* [16,65]. However, ß-glucan was found to significantly increase *Bacteroides* and moderately increase *Prevotella* in individuals with elevated cholesterol levels without showing bifidogenic properties [66].

In contrast to previous findings, BSS, a proxy for intestinal transit time, does not explain variation of the gut microbiota composition in this cohort [41,67]. There were no changes over time in gastrointestinal symptoms in both groups. The gut microbiome composition was significantly associated with the TG, HDL, BMI, , the prebiotic as well as other factors. The finding of the increased TG and BMI are similar to a study by Viera-Silva et al. [20]. Obesity (35.5%), highly prevalent in participants, has been linked with the progression of CKD [68]. Obesity is associated with an alteration in the gut microbiome [69]. In the current study, the TG and weight were positively associated with various organisms. Zeng et al. [70] identified gut microbiota markers for the classification of obesity- related metabolic abnormalities and over 20 genera associated with multiple clinical factors such as weight, waist circumference and lipid abnormalities. TG show associations with gut microbiome biomarkers that may explain some of the changes in the gut microbiome.

Gut dysbiosis in CKD is closely linked to chronic inflammation [57]. CRP was the only measure of inflammation in the current study; it was high in a majority of participants at baseline but did not change over time. The *Bacteroides* 2 enterotype which was highly prevalent in the current study may be contributing to inflammation. It has been found to be lower in statin-treated obese individuals; the mechanistic action for this may be in line with the microbiota-inflammation hypothesis [20]. The action of the ß-glucan fiber may be similar to the lipid-lowering effect of statins and has a positive role to play in the change in the composition of the gut microbiome, at least in CKD patients. Statins have been found to positively correlate with secondary gut-derived bile acids that contribute to the magnitude of LDL-lowering [71]. This mechanism of ß-glucan fiber may also be linked to the action of bile salts; the concentration of bile salts seems to affect the proliferation of bacteria [72,73]. In addition, studies suggest that ß-glucan increase BSH activity, which in turn increase cholesterol excretion and bacteria, such as *Bifidobacterium*, *Bacteroides* and *Lactobaccillus* [74]. There may therefore be different ways that the ß-glucan affects the gut microbiome, through the action of bile salts. The exact mechanisms need tobe further elucidated.

4.1. Strengths of the Study

This single-blind RCT is the first study to examine the effect of the ß-glucan prebiotic on the gut microbiome, uremic toxins and kidney function in parallel in a cohort of participants with CKD not on dialysis using 16S rRNA sequencing methods. It is also the first study to examine enterotypes in this study population as well as the redundancy analysis (RDA) which examined the factors contributing to the gut microbiome variation. In addition, it is the first study to show a high prevalence of *Bacteroides* 2 in the CKD population. Dietary confounders were controlled by using a dietary run-in period, adapted dietary assessment methods and dietary adherence score sheets. Most nutrition status assessment factors remained unchanged during the study, which minimized confounding.

4.2. Limitations of the Study

The study limitations include a large number of participant dropouts from pre-randomization to the end of the study, although every effort was made to contact participants for follow-ups. However, this is reflective of a real-time clinic scenario. The duration of the study may have been too short to see an effect on kidney function and cardiovascular outcomes. Although changes were seen in the gut microbiome, a larger number of participants are probably needed to detect significant shifts from *Bacteroides* 2 to *Prevotella*, as well as shifts in the relative abundances in gut microbiota. The gut microbiome in the two groups would be very similar at baseline due to CKD dysbiosis, and one would need larger sample sizes to detect large variations in these groups compared with healthy individuals where there are distinct differences in the gut microbiome. Dietary intake assessment also has limitations, although the same methodology was applied throughout the study to ensure that the diet intake was adequately assessed and monitored. The lack of a placebo supplement was also a limitation, since patients could not be blinded. However, this did not affect their adherence to the diet.

4.3. Recommendations

A study with a larger sample size and of longer duration would be needed to detect significant changes, especially for studies with a focus on changes within the gut microbiome where inter-individual variability is substantial. Post hoc analysis is recommended for future investigation. Other markers of inflammation should be investigated such as interleukin-1ß, interleukin-6, interleukin-10, tumor necrosis factor, and possibly calprotectin in the stool. The findings of the study suggest that the ß-glucan prebiotic fiber supported by simplified CKD dietary advice favorably affects the gut microbiome. Although this study showed benefits with the ß-glucan prebiotic use, more RCTs are needed before generalized recommendations are made. Owing to the high prevalence of obesity found in the group, other measures should also be sought to address these outcomes, such as weight management, behavior modification and physical activity interventions. SCFAs and bile salt levels should be assessed in future studies to investigate the mechanisms involved in cholesterol-lowering and altered gut microbiota composition. Discount vouchers for healthy, natural foods should be sought for this population. The development of a placebo similar in appearance to the prebiotic with minimal nutritional content or effect on the gut microbiome should be considered. Foods containing natural ß-glucans may be encouraged, this can be found in barley, oats, rye and wheat. Although the results of the study cannot be extrapolated to include these foods, they are high in fibre and could be considered as part of a natural, healthy diet.

5. Conclusions

In conclusion, the current study found that the supplementation of ß-glucan fiber resulted in reduced plasma levels of the free fraction of colon-derived uremic toxins, as well as a favorable change in the composition of the gut microbiome. We therefore reject the hypothesis that kidney function would change with the prebiotic and accept the hypothesis that uremic toxins and the gut microbiome improved with the prebiotic intervention. The uremic toxins correlated with bacteria that were characteristic of gut dysbiosis. There was a high prevalence of *Bacteroides* 2 enterotype and the RDA showed that the prebiotic significantly affected the gut microbiome. Chronic systemic inflammation may be a contributing factor related to the gut dysbiosis in CKD. An increase in uremic toxin levels can contribute to alterations in gut microbiota, resulting in gut dysbiosis, which seems to be exacerbated by metabolic syndrome factors such as obesity and hypertension, which were highly prevalent in the current study population. The mechanism for the changes in this current study may be linked to the lipid-lowering action of the ß-glucan fiber through the action of bile salts and/or attenuation of inflammation, although the only measure of inflammation (CRP) did not show any significant changes.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/nu14040805/s1, Table S1: Generalized expected equations model for biochemical changes over time in the intervention over the control group (proportions), Table S2: Generalized expected equations model for anthropometrical changes over time in the intervention over the control group (means), Table S3: Generalized expected equations model for dietary intake changes over time in the intervention over the control group (proportions), Table S4: Dietary scores for the intervention and control group over time, Table S5: Relative abundances of genera between groups (ALDEx2). Figures S1–S3: Changes in uremic toxins, Figure S4: Consort 10 cohort mapped onto the FGFP background to determine the enterotype for each sample.

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Informed Consent Statement: Informed consent was obtained from all participants involved in the study.

Data Availability Statement: Data is confidential but will be made available upon reasonable requests.

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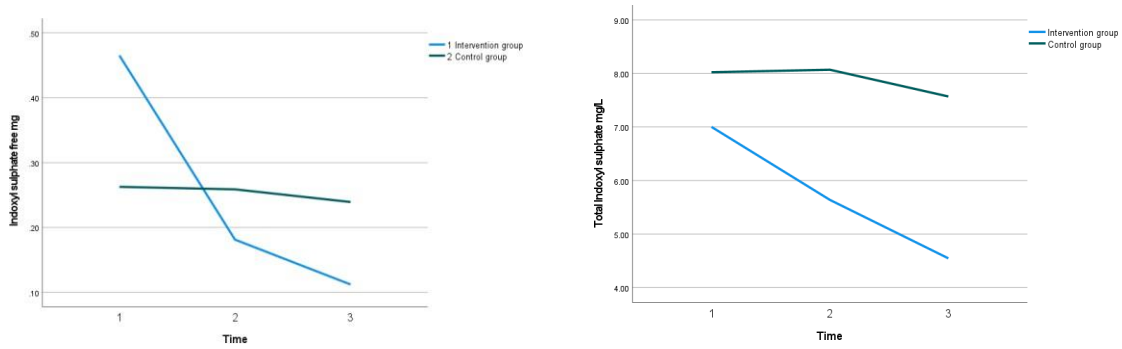
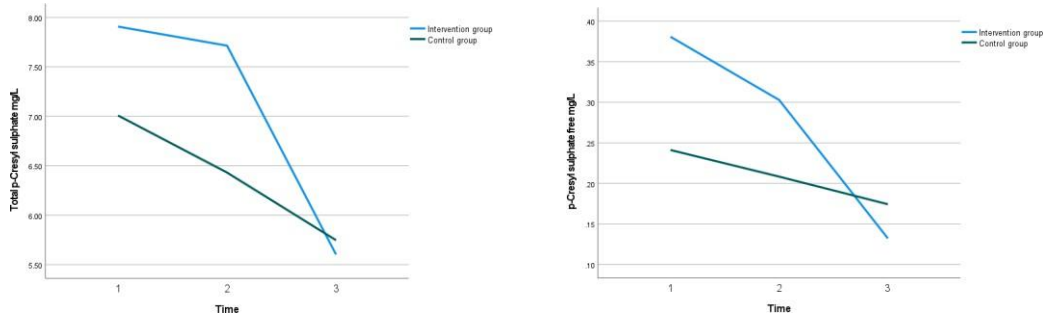
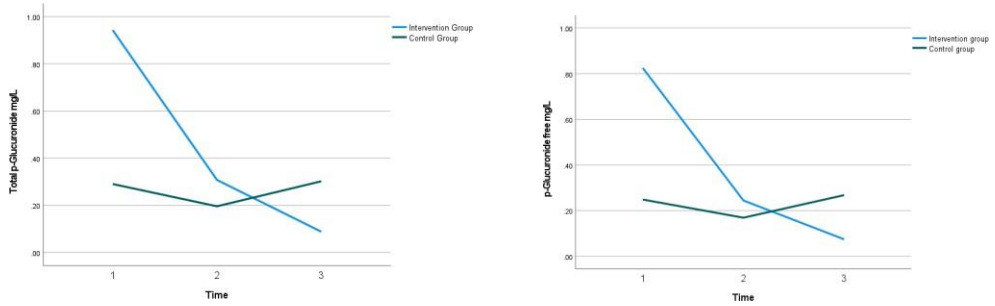
Supplementary data: Tables and Figures



Supplementary Figure S1 : Changes in the Intervention and control group in IxS total and free over time



Supplementary Figure S2: Changes in the Intervention and control group in *p*CS total and free over time



Supplementary Figure S3: Mean changes in the Intervention and control group in *p*CG total and free over time

Supplementary Table S1: Generalized expected equations model for biochemical changes over time in the intervention over the control group (proportions)

	Model 8: Outcome: CRP mg/L			Model 9: Outcome: Potassium mmol/L			Model 10 Outcome: Phosphate mmol/L		
Parameter	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*
[Intervention group]	0.91	0.34-2.41	0.845	0.99	0.92-1.07	0.796	0.99	0.83-1.14	0.714
[week 8]	0.84	0.46-0.51	0.576	0.97	0.92-1.02	0.169	1.03	0.94-1.12	0.575
[week 14]	0.84	0.51-1.38	0.491	1.01	0.95-1.06	0.789	1.03	0.96-1.11	0.379
[Intervention group] *[week 8]	0.96	0.49-1.88	0.912	1.01	0.99-1.01	0.293	1.10	0.96-1.26	0.185
[Intervention group] *[week 14]	2.21	0.60-8.16	0.232	1.00	0.99-1.02	0.593	0.97	0.87-1.09	0.622

Exp: exponential
b = estimated model coefficient; *P* = *P*-value (Wald χ^2 test)
CRP: C-reactive protein

Supplementary Table S2: Generalized expected equations model for anthropometrical changes over time in the intervention over the control group (means)

	Model 1: Outcome: Weight (kg)			Model 2: Outcome: BMI			Model 3: Outcome: Waist circumference (cm)			Model 4 Outcome: MUAC (cm)		
Parameter	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*
[Intervention group]	4.50	-5.95-14.96	0.398	1.78	-1.51-5.11	0.288	5.05	-1.98-13.0	0.150	0.85	-1.73-3.43	0.518
[week 8]	-0.65	-1.34-0.05	0.070	-0.25	-0.50-0.10	0.057	-0.85	-1.74-0.31	0.059	-0.20	-0.58-0.17	0.291
[week 14]	-0.84	-2.05-0.39	0.181	-0.33	-0.78-0.11	0.147	-0.75	-1.75-0.25	0.142	-0.25	-0.78-0.28	0.362
[Intervention group] *[week 8]	0.88	-1.07-1.25	0.882	0.02	-0.48-0.49	0.985	-0.46	-1.78-0.86	0.492	-0.06	-0.68-0.56	0.840
[Intervention group] *[week 14]	0.43	-1.35-2.21	0.633	0.15	-0.56-0.87	0.678	-0.96	-2.70-0.80	0.286	-0.45	-1.32-0.35	0.252

Exp: exponential
b = estimated model coefficient; *P* = *P*-value (Wald χ^2 test)
BMI: body mass index

Supplementary Table S3:
Generalized expected equations model for dietary intake changes over time in the intervention over the control group (proportions)

	Model 1: Outcome: Energy (kcal)			Model 2: Outcome: Protein (g)			Model 3: Outcome: Total fat (g)			Model 4: Outcome: Saturated fat (g)			Model 5 Outcome: Diet fibre (g)			Model 6 Outcome: Potassium (mg)			Model 7 Outcome: Phosphate (mg)			Model 8 Outcome: Sodium (mg)		
Parameter	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*	Exp(b)	95% CI	*P*
[Intervention group]	0.96	0.80-1.17	0.693	1.10	0.89-1.3	0.375	0.98	0.77-1.25	0.858	1.08	0.83-1.40	0.556	1.05	0.88-1.24	0.603	1.05	0.88-1.26	0.541	1.12	0.91-1.34	0.282	0.72	0.71-1.28	0.787
[week 8]	0.94	0.83-1.06	0.312	0.93	0.80-1.10	0.407	0.85	0.72-1.00	**0.035**	0.88	0.74-1.04	0.135	1.10	0.98-1.22	0.093	1.00	0.90-1.11	0.945	0.96	0.83-1.11	0.592	0.70	0.70-1.11	0.297
[week 14]	0.90	0.76-1.08	0.266	0.95	0.78-1.15	0.601	0.84	0.64-1.10	0.203	0.83	0.63-1.10	0.206	1.10	0.95-1.24	0.219	1.02	0.88-1.20	0.768	0.97	0.80-1.12	0.822	0.62	0.62-1.16	0.322
[Intervention group] * [week 8]	1.00	0.84-1.19	0.990	0.96	0.80-1.17	0.695	1.08	0.87-1.34	0.455	0.78	0.80-1.21	0.833	0.91	0.78-1.07	0.294	0.96	0.83-1.11	0.962	0.95	0.80-1.13	0.555	0.73	0.73-1.44	0.879
[Intervention group] * [week 14]	1.08	0.85-1.36	0.530	0.98	0.78-1.23	0.903	1.15	0.82-1.60	0.403	0.80	0.80-1.57	0.501	0.93	0.77-1.12	0.458	0.93	0.76--1.13	0.934	0.96	0.76-1.22	0.761	0.80	0.81-1.86	0.341

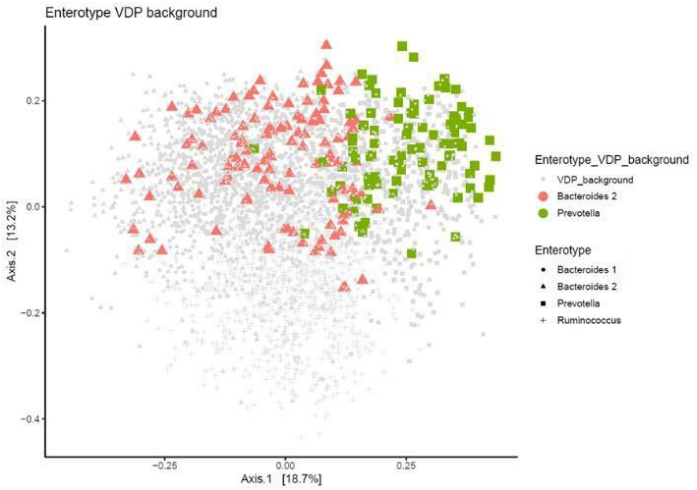
Exp: exponential
b = estimated model coefficient; *P* = *P*-value (Wald χ^2 test)

Supplementary Table S4 : Dietary adherence scores for the intervention and control group over time

Group	Baseline (%)	4 weeks (%)	Week 8 (%)	Week 14 (%)
Intervention	89.4	93.3	94.6	94.2
Control	85.6	92.3	91.0	91.7

Supplementary Table S5 Relative abundances (ALDEX2)

Genera	Adj p value (Welch)	Adj p value (Wilcoxin)	Relative abundance overall	Relative abundance within Control	Relative abundance within Intervention	Difference between groups	Difference within groups	Effect size	Overlap between groups
Prevotella	0.27	0.36	3.23	2.29	3.70	1.03	7.96	0.12	0.44
Faecalibacterium	0.63	0.68	6.36	6.36	6.35	0.11	2.12	0.04	0.48
Roseburia	0.14	0.04	5.54	5.92	5.12	-0.77	2.84	-0.23	0.38
Blautia	0.46	0.93	5.47	5.37	5.52	0.03	1.78	0.02	0.49
Bifidobacterium	0.74	0.93	1.94	1.69	2.21	-0.13	6.51	-0.02	0.49
Catenibacterium	0.51	0.61	1.55	1.70	-1.16	-0.34	8.87	-0.04	0.48
Bacteroides	0.75	0.47	5.18	5.44	4.87	-0.39	3.52	-0.10	0.45
Ruminococcus	0.55	0.72	4.33	4.39	4.28	-0.19	2.51	-0.05	0.47
Ruminococcus 2	0.62	0.75	4.15	4.20	4.11	-0.10	2.00	-0.04	0.48
Dorea	0.20	0.27	3.74	3.85	3.67	-0.32	1.86	-0.14	0.43
Holdemanella	0.85	0.81	3.11	3.12	3.10	-0.19	7.76	-0.03	0.48
Fusicatenibacter	0.91	0.86	3.04	2.93	3.15	0.09	2.55	0.03	0.49
Coprococcus	0.88	0.84	3.48	3.49	3.48	-0.09	2.14	-0.03	0.48
Clostridium _IV	0.56	0.33	3.38	3.63	3.17	-0.31	2.15	-0.12	0.44
Clostridium _XIVa	0.37	0.37	2.63	2.45	2.92	0.39	2.99	0.11	0.44



Supplementary Figure S4: Consort 10 cohort mapped onto the FGFP background to determine the enterotype for each sample.

CHAPTER 8: DISCUSSION

8.1 Background to the conceptualisation of the study

The aim of this research project was to investigate the effect of a ß-glucan prebiotic supplement on kidney outcomes, uraemic toxins and the gut microbiome in predialysis chronic kidney disease (CKD) participants.

The modulation of the gut microbiome as a therapeutic option to improve renal outcomes was an innovative area that inspired the conceptualisation of the study. The gut microbiome plays an important role in maintaining host homeostasis with regard to normal gut integrity, immunological functions and nutrient interactions. It is therefore considered to be an essential organ [1]. Gut dysbiosis involves an alteration in the function and composition of gut microbiota and has been associated with CKD [2]. Increasing evidence suggests that gut dysbiosis drives CKD progression through various mechanisms, including intestinally derived uraemic toxins and immune-mediated factors, as well as neurohormonal-mediated factors. Probiotics, prebiotics and synbiotics seemed to be the most researched supplements in the modulation of the gut microbiome. Most of the research at the time of conceptualisation showed promising results of prebiotics in improving kidney function [3, 4] and uraemic toxins [5, 6], while evidence for changing the gut microbiome was limited [7]. Probiotics were not considered for the study, since there are potential concerns with their being used on their own in CKD [8]. Positive results were also found with synbiotics in altering uraemic toxins [9, 10] and the gut microbiome [9]; however these were especially formulated for the studies and would take long to formulate and be too expensive to consider in our context. Oats was considered as a natural option and seemed to be the most promising in terms of the beneficial properties of ß-glucan in altering the gut microbiota [11, 12] and uraemic toxins [13], albeit in healthy individuals. The product GlucaChol-22® contained the ß-glucan in much higher concentrations and was easier to incorporate in the diet with about 1.5 scoops a day, which is equivalent to many bowls of oats. This product was therefore used in the study owing to its ease of consumption and its beneficial properties. After critically evaluating the research in the area, it was decided that the ß-glucan prebiotic would be the optimal supplement from a mechanistic as well as a practical perspective within the South African setting.

8.2 Contextualisation of the study findings

This is the first study to simultaneously investigate the effect of a prebiotic on all three outcomes of the study, namely, kidney function, uraemic toxins and the gut microbiome. The only other study that investigated these outcomes simultaneously used a synbiotic product [9].

A four-week pre-randomisation period was used to determine descriptive data of patients with CKD. It also provided the participants with the opportunity to become familiar with the dietary guidelines advised to ensure that dietary changes were not seen as an additional study variable that influenced the intervention outcome. Three of the four objectives were investigated during this phase.

Objective 1

The first objective was to describe the baseline characteristics of the gut microbiome profile and uraemic toxins in CKD patients on admission to the study.

I am a qualified dietitian specialising in nutrition in CKD. Therefore, investigating kidney disease and associated outcomes was not problematic. However, the analysis of uraemic toxins and the gut microbiome proved to be more challenging. The analysis methods of the uraemic toxins could not be replicated in any laboratories in South Africa, while local methods for gut microbiome analysis were not reliable and very expensive. This created an opportunity for collaboration with international researchers to analyse these outcomes expertly as well as fostering international collaborative links. The uraemic toxins were analysed by Prof. Glorieux and her team at the Ghent University Hospital in Belgium, while the gut microbiome analysis was performed by Prof. Jeroen Raes and his team at the VIB laboratories at KU Leuven, Belgium. I had the privilege of visiting both these institutions to observe the methods to be used in the study; however due to Covid-19, could not visit again to observe the analysis of my own samples. This necessitated many virtual meetings with both teams to discuss the analysis and interpretation of the results.

The baseline gut microbiome analysis showed that there was a high prevalence of the *Bacteroides* 2 enterotype in the participants, even higher than found in other studies

[14]. The gut microbiome analysis is described in Chapter 7 of this dissertation. *Bacteroides 2* and *Prevotella* were the only enterotypes found in participants, compared with the Flemish Gut Flora project which showed four enterotypes [15]. *Bacteroides* 2, which is characterised by a high proportion of *Bacteroides* and low proportion of *Faecalibacterium* and cell densities, has been associated with inflammatory conditions [14]. The uraemic toxins at baseline were higher than values in healthy individuals; however, they did not change significantly from pre-randomisation to randomisation.

Objective 2

Objective 2 set out to describe the biochemical profile and nutritional status on admission to the study.

The pre-randomisation nutritional status of participants was published in *Nutrients* in November 2020. This is described in Chapter 5 and linked to Objective 2 of the study [16]. This paper investigated the pre-randomisation nutritional status of the predialysis CKD patients on enrolment and before they were randomised to the intervention or control group. Seventy participants entered the study. The mean age of the participants was 41.7 ± 11.8 years, with a slight predominance of females (53%). Most participants were unemployed, earning less than US$126 per month and had up to Grade 11 schooling. A very high prevalence of overweight (30%) and obesity (36%) was found. Abdominal obesity was found in 60% of participants. A low prevalence of undernutrition (3%) was found. Biochemistry showed high urea and creatinine, and a low GFR, reflective of CKD. The CRP was high in 60% of participants. Dietary assessment showed an unhealthy dietary pattern with a high sugar, saturated fat and protein intake.

Objective 3

The third objective set out to educate participants on the CKD diet in the pre-randomisation phase and evaluate dietary adherence based on the dietary education.

While working on the logistics of the project, I found that there was a lack of suitable materials to use in the dietary education run-in period. The available materials were based on traditional guidelines that include many dietary restrictions such as protein,

phosphate, sodium and potassium. Literature reports a lack of evidence for the traditional guidelines, with many authors advocating less restrictive dietary advice in CKD based on dietary pattern studies [17–21]. These studies show that diets that are rich in fruits, vegetables and wholegrains have better outcomes in CKD compared with diets that are higher in processed, fried and take-away food. After reviewing the literature and in consultation with renal expert colleagues, an infographic was developed encouraging a natural diet, while limiting processed foods. It was subsequently published in the *Journal of Renal Nutrition* and presented as the first article of this PhD dissertation [22]. This paper, discussed in Chapter 4, was linked to the third objective of the study.

Subsequent to the publication of the infographic, I was approached by the International Society of Renal Nutrition and Metabolism (ISRNM) to serve on their educational committee and to present a webinar on the infographic. The infographic is currently being used at two academic hospitals in South Africa for patient education and was also used as an intervention in a master's study I supervised. Although this infographic was developed to be used in the study, the outcomes of the publication and the effect on the CKD community were much broader. It allowed for collaboration with national and international dietitians and assisted in the education of CKD patients. I was approached after the ISRNM webinar to assist with a translation of the infographic into Spanish for colleagues in Chile. It was also translated into isiXhosa and Afrikaans.

Participants were advised to follow a CKD diet using a simplified dietary infographic at the pre-randomisation phase and throughout the study, encouraging a natural diet and avoiding additives and processed foods. A protein restriction of 0.8 g/kg was advised and a portion list of foods was given to meet the protein prescription. The KDOQI guidelines were subsequently updated late in 2020 [23], which was after data collection was completed. The recommendations for protein and other nutrients used in this study were therefore based on the older guidelines. The effect of the dietary intervention was described in Chapter 6 of this dissertation.

The main findings of Chapter 6 were that the dietary intervention lowered many nutritional parameters after four weeks compared with their baseline values. This

paper was published in *SAJCN* in February 2022. There were 59 participants that were randomised. The socio-demographics were similar to the pre-randomisation demographics. There was a significant reduction with regard to anthropometry BMI ($P < 0.006$) and waist circumference ($P < 0.001$). Almost all dietary intake variables were significantly reduced and there was a high dietary adherence. Total serum cholesterol ($P < 0.045$) and triglyceride levels ($P < 0.017$) were also reduced. Uraemic toxins and kidney function remained unchanged. With regard to the gut microbiome, there were no significant changes in relative abundances in the gut microbiome from pre-randomisation to randomisation. This simplified dietary infographic resulted in improved nutritional outcomes, with no change in uraemic toxins or gut microbiome. The lack of change in uraemic toxins and gut microbiome may be considered a positive finding, since these worsen as CKD progresses. Dietary adherence was high, although it was the lowest for the fruit and vegetable groups, despite being advised to incorporate these into the diet.

Objective 4

The main aim of the study, which was the fourth objective of the study, was to conduct a single-blind RCT to compare the effect of ß-glucan prebiotic fibre on the following outcomes in the intervention (CKD diet and ß-glucan prebiotic fibre) and control group (CKD diet only) at the baseline visit (randomisation), week 8 and week 14: gut microbiome profile, kidney function, uraemic toxin levels and other biochemical parameters including lipid function tests and inflammatory markers. Participants could not be blinded, since the control group remained only on the CKD diet.

Chapter 7 describes the randomised control trial of the effect of the ß-glucan prebiotic on the gut microbiome, uraemic toxins and kidney function and was published in *Nutrients* in February 2022. The main findings were that there was no significant change in kidney function over time. There was a significant reduction in LDL cholesterol at week 8 ($P < 0.037$); however, this effect disappeared at week 14. There was a significant reduction in uremic toxin levels at different time points, in free IxS at 8 weeks (exp b: 0.46, CI: 0.28 – 0.76, $P = 0.003$) and 14 weeks (exp b: 0.35, CI: 0.21– 0.60, $P < 0.001$), free *p*CS (exp b: 0.48, CI: 0.29 – 0.82, $P = 0.006$) at 14 weeks and total and free *p*CG (exp b: 0.14, CI: 0.08 – 0.28, $P < 0.001$, exp b: 0.13; CI: 0.06 – 0.27, $P < 0.001$, respectively) and at 14 weeks.

With regard to the gut microbiome, the main enterotypes that were found were *Bacteroides* 2 and *Prevotella*. The prevalence of *Bacteroides* 2 was high.

The redundancy (RDA analysis) is the cumulative effect size of the covariates affecting the compositional variation of the gut microbiome. The redundancy analysis showed a few factors significantly affected the gut microbiome: these included triglyceride levels ($P < 0.001$), cause of renal failure ($P < 0.001$), gender ($P < 0.001$), body mass index ($P = 0.002$), high-density lipoprotein ($P < 0.001$) and the prebiotic intervention ($P = 0.002$). There were trends in changes in the relative abundances in the intervention group, specifically, increases in *Prevotella* and *Faecalibacterium*, while *Blautia* and *Bacteroides* were reduced; however, these were not significant when correcting for multiple testing. When looking at the enterotypes, there was a trend in a reduction in *Bacteroides* 2 in both the intervention group (from 60% to 45%), whereas it increased in the control group (from 50–60%), although this was not significant (AldeX2 = 0.298). There were correlations with uraemic toxins reflective of gut dysbiosis as well as correlations with triglyceride levels, weight and body mass index (BMI). Most of the outcomes in this study were positive findings, with two of the main outcomes being affected by the intervention of this current study.

8.3 Contributions to knowledge and implications for practice

When analysing the results of this study from the information presented above, there were a few findings that differ to current research in CKD. The contribution to knowledge and implications for practice will be discussed according to the findings of the study. In the current study, most of the participants were overweight and obese, mostly in stage 5 of kidney failure and had an unhealthy dietary intake at the pre-randomisation phase. CKD patients already present with obesity and hypertension at their diagnosis of CKD. Obesity, together with clinical conditions associated with the metabolic syndrome, have an altered gut microbiota associated with inflammation [24]. The metabolic syndrome may be a new challenge in CKD, instead of undernutrition. *Bacteroides* 2 and *Prevotella* were the two enterotypes identified in the study, with *Bacteroides* 2 having a high prevalence.

The high prevalence of obesity and hypertension in the current study may be a contributor to the changes in the gut microbiome in CKD participants; this has not been

investigated previously and provides some new insights into additional causes of gut dysbiosis in CKD. The prevalence of obesity is high in South Africa and is a contributing factor to the progression of CKD [25, 26]. The high prevalence of obesity in CKD was also reported by others [27, 28]; on the other hand, other studies reported high prevalences of undernutrition [29, 30]. It may be that the nutritional status in CKD participants in this study is reflective of a younger and more obese background population, compared with other studies. The dietary intake of the study population was also reflective of an unhealthy dietary pattern which may have contributed to the obesity. Other studies report on insufficient energy and protein intakes in this population [27, 31].

The dietary intervention, using the simplified dietary infographic and counselling by the dietitian, resulted in significant nutritional changes in weight, BMI, waist circumference, total cholesterol and triglyceride levels, and nearly all dietary variables, while uraemic toxins remained unchanged from the pre-randomisation to the randomisation visit. Once participants were randomised, these dietary changes were sustained. Dietary adherence was high, although lowest for the fruit and vegetable group. While it seems that dietary advice had some effects on nutritional changes, kidney function, uraemic toxins and the gut microbiome did not significantly change, although some of these outcomes had a decreased trend, while others remained stable. The simplified approach resulted in a higher adherence to the 6-tips diet [32]. The 6-tips diet study, also based on simplified guidelines (although different guidelines from the current study), advised in stage 3 to 5 CKD participants, resulted in improved dietary adherence and metabolic parameters. Both these studies provide evidence for the simplification of the diet in CKD. Dietary changes can be made by substituting healthier foods for unhealthy foods and this does not necessarily make the diet more expensive.

However, once the prebiotic was introduced, there were significant changes in uraemic toxins and gut microbiome composition. Rossi et al. [9] and Guida et al. [33] found a reduction in *p*CS in CKD and haemodialysis participants respectively, who consumed a synbiotic containing different strains of *Bifidobacterium, Lactobacilli and Streptococcus* prebiotics for four weeks. Lopes et al. [10] also found a reduction in *p*CS and IxS by consuming sorghum as a prebiotic and *Bifidobacterium* as a probiotic in haemodialysis participants for seven weeks. The finding of the reduction in IxS in CKD

predialysis participants has not been reported on previously. It may be due to the effect of the ß-glucan, since this is different from the inulin and the other prebiotics used in the other studies.

The changes in the gut microbiome were influenced mainly by serum triglycerides, BMI and the prebiotic intervention. It may be that the change in the gut microbiome could be due to the trend in the shift in *Bacteroides* 2 to *Prevotella*, and may be linked to the lipid-lowering action of the prebiotic. Gut dysbiosis in CKD is caused by many factors, as previously discussed [2]. This contributes to the high levels of uraemic toxins, since they are intestinally derived [1]. This bidirectional relationship is characterised by high levels of inflammation and contributes to increased cardiovascular risk [34].

The results of this study were different from gut microbiome changes described by Rossi et al. [9]. They did not investigate enterotypes or RDA associations linked to the gut microbiome. They did, however, find significant changes in *p*CS and levels of *Bifidobacterium*, while there was an increased trend in *Lactobacilli*. It may be that the increase in the *Lactobacilli* and *Bifidobacterium* was linked to the addition of these in the synbiotic. The ß-glucan supplement benefits in this study were linked to trends in changes in *Prevotella*, *Bacteroides* and *Faecalibacterium*, in contrast to Rossi et al. [9] On a practical level, although dietitians are important in advising on dietary changes, it seems additional therapy beyond the CKD diet is required to alter uraemic toxins and the gut microbiome, and in this instance ß-glucan prebiotic fibre resulted in some positive changes. It may be advisable that CKD patients use this supplement in addition to their diets to improve their gut dysbiosis and all the factors linked to it after more research has been conducted. The supplement is made from oats, therefore advising oats and foods that promote the production of short-chain fatty acids (SCFAs) may assist further in altering the gut microbiome if prebiotic supplements are not affordable. A shift towards a more plant-based diet may also help in this regard. Plant-based diets have been associated with positive gut microbiome changes and a reduction in uraemic toxins [35].

This mechanism for the findings in the current study may be linked to the lipid-lowering action of the (SCFAs) [36] and possibly the attenuation of inflammation which may have

affected the uraemic toxins and gut microbiome. Obese individuals treated with statins have shown a lower prevalence of *Bacteroides* 2 in the statin-treated group; the authors hypothesised this may be due to the modulation of the gut microbiota or through the development of enterotypes not associated with inflammation [37]. The cholesterol-lowering action of ß-glucan may be affected by other mechanisms besides the gel-forming properties and a reduction in bile and cholesterol absorption. The action of bile salt hydrolase (BSH) may play a role in the mechanism of action of cholesterol lowering; it has been hypothesised that increased BSH activity due to bacterial modulation could reduce plasma cholesterol levels [11]. These factors have not been investigated in any other studies related to the modification of the gut microbiome.

In conclusion, this study in its entirety had a few novel findings. A high prevalence of overweight and obesity, other nutritional abnormalities and an unhealthy diet was found in participants at the pre-randomisation visit. A simplified infographic was developed and used to educate participants during the study period due to a lack of suitable educational materials. The dietary intervention which was done 4 weeks pre-randomisation and continued throughout the study, resulted in significant changes in the nutritional status, while uremic toxins remained stable. There was a favourable effect of the ß-glucan prebiotic fibre on the gut microbiome and uraemic toxins in CKD participants, with no effect on kidney function. We therefore reject hypotheses 1 and 3 and accept hypothesis 2.

8.4 References:

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CHAPTER 9 : CONCLUSION

Conclusions are drawn by revisiting the research question: How does the inclusion of ß-glucan prebiotic fibre (oats) in addition to a CKD diet affect the gut microbiome, uraemic toxins and kidney function of CKD predialysis patients over 14 weeks compared with the CKD diet alone?

The results of this randomised controlled trial (RCT) show that the ß-glucan prebiotic fibre (oats), in addition to a CKD diet, significantly affected uraemic toxins and the gut microbiome, while not affecting kidney function. The diet, advised at pre-randomisation, resulted in nutritional status changes while not affecting kidney function, uraemic toxins or the gut microbiome. From a practical perspective, it can be argued that no change in these outcomes is a positive finding, since they worsen over time as kidney function declines. Once supplementary fibre was added to the diet already being followed, it resulted in significant changes in uraemic toxins and the gut microbiome. While the prebiotic was found to be a significant factor in changing the gut microbiome, the relative abundances did not show any significant changes. This may be due to a trend in the reduction of the inflammatory *Bacteroides* 2 in the intervention group; however larger numbers are needed to show significance in this shift. Uraemic toxins correlated with gut microbiota reflective of organisms found to be higher or lower in CKD compared with healthy individuals. Other metabolic factors such as triglycerides, BMI and lipids also significantly affected the gut microbiome. The prevalence of obesity and hypertension was also high in this study group. These factors may also provide some new insights into the causes of gut dysbiosis in CKD.
While the mechanism of the ß-glucan prebiotic fibre may be related to the gel-forming properties of the ß-glucan prebiotic fibre lowering cholesterol, it could also relate to bile acids and possibly the attenuation of inflammation, since it may be that these properties result in more favourable enterotypes.

The study resulted in many skills being developed, including development of new educational material which was used locally and internationally; development of an understanding of new methods of analysis such as uraemic toxin and the gut microbiome, which are outside the scope of nutrition; applying for funding for the research; performing statistical procedures under the supervision of biostatisticians; using a new program especially developed for the visualisation of the gut microbiome results; submission of

journal articles to journals; presenting at local and international congresses; collaboration with international researchers; and finally, the academic process of writing up the dissertation. I performed an RCT in a novel area of nutrition, with results that have practical implications, while also developing numerous skills in the process.

Limitations of the study

The limitations of the study include the high dropout rate, despite efforts to contact the participants to return. Some participants inevitably had to exit the study owing to the initiation of dialysis. The short study period was a limitation, since the effect of the prebiotic on kidney function and cardiovascular outcomes would need a longer study period to show an effect. The sample size was too small to detect significant changes in the shifts of enterotypes and relative abundances, and the sample size calculation should have been based on changes in other gut microbiota. The use of the QFFQ can be highly accurate, although measurement errors related to methodology remain. The use of the diet-adherence scores were also subject to the participants' responses and bias towards answering "correctly'. Under-reporting of dietary intakes is a limitation of the study. Also, the food composition tables used for analysis of the dietary intake are based on an average intake of nutrients, hence the nutrient intake reported may have some inaccuracies. Compliance was difficult to measure since some participants forgot to return their containers. There may have also been a larger difference in the effect of the prebiotic between the intervention and the control group if the control group had not been advised on the diet. However, the whole group was advised on the CKD diet in the pre-randomisation phase. The reason for the education at this phase was to ensure that the diet was stabilized throughout the study, so that any changes in outcomes was ascribed to the ß-glucan prebiotic and not the diet. The effect of the ß-glucan prebiotic may also have been minimized if the diet was not changed. Additionally, it would not have been ethical to have the two groups on different diets. The control group therefore also benefited from the dietary education rather than just continue with usual care.

Recommendations for future research

- It is recommended that further studies be performed, using a larger sample size and over a longer period. Additional measures to retain participants should be considered.

- Post-hoc analysis will include further investigation of the metabolic syndrome and consequences in the study participants, whether medications has any effect on the prebiotic as well as whether there were any significant differences of the effect of the prebiotic at the different stages of CKD.
- It is recommended that the infographic be used during dietary education for CKD participants since there were many advantages of using the infographic. It was easy to follow, understand and allowed for dietary changes to be made. It allowed participants in both groups to realise that their diets does'nt have to be as restrictive and that they can have a greater variety of food. It was also not necessarily more expensive for them to adapt their current diets.
- Additional outcomes such as bile salts and short chain fatty acids should be measured, as this offers another novel area of investigation into the mechanism behind prebiotics and the modulation of the gut microbiome in CKD. Additional inflammatory markers should also be investigated, since the current study did not show any change in C-reactive protein (CRP) during the study.
- The inclusion of oats in the CKD diet should be recommended; even though the ß-glucan may be present at a lower dose in oats, it may confer some benefits. Collaboration with the manufacturers of the ß-glucan to offer the product at a reduced rate to CKD patients should be considered, but perhaps only after more RCTs have been performed. The current cost is R13 per day, if the rate can be subsidised, it may be sustainable for patients to use.
- Similarly, discount vouchers for healthy, natural foods should be sought for this population due to their lower socio-economic status, perhaps in conjunction with the National Kidney Foundation of South Africa. Networking with supermarket chains to offer discounts on healthy foods such as fruit, vegetables, wholegrains and lean proteins, ie two for the price of one or a percentage off purchases may be some of the options to investigate. This may need the involvement of a multi-disciplinary team on a governmental, public and private level. Perhaps some of the governmental sugar tax earnings could be channelled into initiatives such as food vouchers and subsidising the prebiotic. Patients could then access these vouchers when going for follow up visits at the hospital or clinic to be used at a supermarket convenient to them. Governments should work with business to distribute food to the vulnerable populations. Food reserves should be monitored

safely and released when prices spike. Open food markets should be encouraged. Education around sustainable food gardens should be encouraged. This may also be beneficial in the current COVID-19 pandemic, where many people have lost their jobs and where food security is a major concern.

Reflections of the PhD journey

As an avid runner, I could relate my journey as training preparation for a marathon: the conceptualisation, data collection, data entry, data analysis, writing and submission of articles to journals being the training races with the final marathon being the final dissertation. I could relate to the perseverance, hard work, dedication, critical thinking and sacrifice needed to complete the journey. Sometimes I hit a brick wall, but learned to find the strength to continue. Some ‘races’ were better than others. Along the way, I developed many professional skills, travelled, met amazing people, developed beautiful friendships, and had friends, colleagues and family cheering me from the side. The COVID-19 pandemic added many challenges during the data analysis and writing up of the dissertation. The CKD community is a special community, and I shall always love working in this field. The journey has allowed me to develop on several levels, having progressed as an academic, individual and researcher.

ADDENDA

ADDENDUM A: CONSENT FORM

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:

The Effect of ß -glucan Prebiotic Fibre (Oats) on the Gut Microbiome of Chronic Kidney Disease Patients (Stage IV and V) and its Impact on Kidney Function.

REFERENCE NUMBER: S18/03/064

PRINCIPAL INVESTIGATOR: Mrs Z Ebrahim

ADDRESS: 3rd Floor, Clinical Building, Division of Human Nutrition, Faculty of Medicine and Health Sciences, Stellenbosch University.

CONTACT NUMBER: 0219389137

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

- The project aims to investigate how certain foods you eat will affect your gut function and how this in turn may influence the progression of your chronic kidney disease. The gut consists of protective (good) and harmful (bad) bacteria. Certain foods, like fibre, may increase the number of good bacteria. We want to determine if increasing the number of goodbacteria will decrease the build-up of toxins in your body that may cause kidney disease to progress to end stage kidney disease.
- This study will take place at the Renal Unit, Tygerberg Hospital only. We will enrol 70 patients in total, divided into two groups of 35 each. Both groups will be placed on a diet for kidney disease and one group will also receive an oats fibre supplement to take additionally.
- You will be assigned to either the diet only or diet plus oat fibre supplement group. This will be done randomly using a list generated by a computer. The computer generates a list of numbers and you will be assigned according to the number that you receive in your patient allocation envelope to either group. This list is confidential, so no one will know which group you will be allocated to until the envelope is opened.
- You should continue with your usual medications as prescribed by your doctor.

Why have you been invited to participate?

- You have been asked to participate since you are a patient managed by the Tygerberg Hospital renal unit with chronic kidney disease, and you are not yet on dialysis treatment.

What will your responsibilities be?

- You need to provide permission for the study team to have access to your medical records to obtain information about you medical condition and medications.
- You will be assessed at your clinic appointment as your first assessment and then follow-up appointments will be at week 4, 8 and 14.
- You will be assessed for you weight, height and other body measurements at the first and follow up assessments at week 8 and week 14..
- You will be asked to follow a diet for your disease condition for one month, thereafter you might be asked to either continue with the diet alone only or in addition, take an oat fibre supplement. This supplement needs to be taken daily in prescribed quantities.
- You will be expected to come for follow up appointments (± 45- 60min) for the duration of the study, at your initial visit and at week 4, and 14 you will be expected to give a stool sample, have your bloods (± 2 teaspoons of blood) taken by the nurse and have a dietary assessment which will be a few questions about how you are following your prescribed diet.There will be shorter visits (± 20 minutes) at week 8 to see how you are following your prescribed diet and to issue the supplement if you are receiving it.
- All the necessary kits for stool sample collection will be provided to you.
- You will have to freeze the stool sample after you have collected it and bring to your appointments.
- You will have to return your empty containers of the oats supplement used (if issued to you) at every visit.

Will you benefit from taking part in this research?

- You will benefit by receiving dietary advice that is suitable for your disease condition which may improve your symptoms. If you do receive the fibre supplement, this may improve the good bacteria in your gut which may reduce the toxins from your kidney failure and slow down the disease progression
- If you do not receive the product during the study, you will receive some samples when the study has been completed if the product has been shown to be of benefit for patients with your condition.

Are there in risks involved in your taking part in this research?

- There may be slight gut discomfort if you receive the fibre supplement, but this side effect will be minimal since we will slowly introduce the supplement into the diet.
- There might be slight pain and discomfort when the nurse draws your blood.

If you do not agree to take part, what alternatives do you have?

- If you do not agree to participate this will not affect you in any way, you will continue with your usual clinic care.

Who will have access to your information?

- Only the investigator, her supervisors and the statistician of the study will have access to your information. All the information with remain confidential and your identity will be protected. We will not record your name on any computer databases.

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

- It is not expected that you will be injured by participating in this project.

Will you be paid to take part in this study and are there any costs involved?

- You will be reimbursed for your time, inconvenience and travel to take part in the study for each study visits, at your initial visit and follow up visits. There will be no costs involved for you, if you do take part. You will be given a R100 shopping (local supermarket) for your time and R50 cash for your transport for every visit.
- The fibre supplement will be given free of charge.

Is there anything else that you should know or do?

- You can contact Mrs Z Ebrahim at tel 021 938 9137 if you have any further queries or encounter any problems.
- You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- You will receive a copy of this information and consent form for your own records.
- Your stool and a small amount of your blood samples will be sent to another facility to be analysed due to the test not being performed at the current facility.

Declaration by participant

By signing below, I..... agree to take part in a research study entitled *(*The Effect of - ß glucan Prebiotic Fibre (Oats) on the Gut Microbiome of Chronic Kidney Disease Patients (Stage IV and V) and its Impact on Kidney Function).
I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.
- I understand that my blood and stool samples will be sent away for analysis to another facility due to the tests not being done at the study facility.

Signed at (*place*) .....…..…….. on (*date*) ……...……..

.....	.....
Signature of participant	**Signature of witness**

Declaration by investigator

I *(name)*..... declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…….. on (*date*).....2018.

....
Signature of investigator **Signature of witness**

Declaration by interpreter

I *(name)*..... declare that:

- I assisted the investigator (*name*) .....to explain the information in this document to (*name of participant*) ……..…….. using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…….. on (*date*).....(2018)

....
Signature of interpreter **Signature of witness**

ADDENDUM B: FOOD FREQUENCY QUESTIONAIRE

Participant number:_____

Usual intake during the last month														
Column 1		**Column 2**							**Column 3**					
		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
1	RED MEAT: low fat cuts (no visible fat) e.g. Steak, lean minces, roast, ham (beef, mutton, pork)								90g/ 3X ▭					
2	RED MEAT: high fat cuts e.g. Regular mince, ribs chops with fat (beef, pork, mutton)								90g/ 3X ▭					
3	MEAT STEW: e.g. chuck, neck or pieces (with or without vegetables) Beef pieces								1 cup or 2X ◎					
4	CHICKEN/TURKEY: with skin								90g/ 3X ▭					
5	CHICKEN/TURKEY: without skin								90g/ 3X ▭					
6	FRIED CHICKEN/ : Kentucky or homemade								90g/ 3X ▭					
7	FISH: fried in fat/oil								90g/ 3X ▭					
8	FISH: steamed, grilled Hake, tuna Snoek, salmon								90g/ 3X ▭					
9	FISH: tinned sardines, pilchards								90g/ 3X ▭ or ½ small tin					
10	ORGAN MEATS: liver, kidney, heart, tripe (beef, sheep, chicken)								90g/ 3X ▭					
11	BACON								1 rasher					
12	EGGS (WITHOUT FAT): boiled/ poached								1 egg					
13	EGGS (WITH FAT): scrambled/ baked/ omelette								1 egg					
14	Fried/ not fried Viennas, russians, Frankfurt etc.								10cm sausage					
15	Hamburger, sausages, boerwors								10cm 90g/ 3X ▭					
16	Polony, salami, ham								2 slices					
17	Fried/grilled oven Crumbed chicken Crumbed fish Chicken schnitzel								90g/ 3X ▭					
18	PIE:Chicken/beef								1X ◎ or 1 med					

		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
19	LOW FAT (FRESH/ POWDERED): milk, sour milk (Maas) on porridge/cereal/TEA or to drink (excluding milk blends)								½ cup or ½ std glass					
20	COFFEE CREAMER IN TEA/COFFEE: eg. Cremora								1 tsp					
21	YOGHURT: full cream/LOW FAT								½ cup or single serving					
22	ICE-CREAMS (DAIRY/Non dairy): ice-cream								1 cup					
23	CHEESE, CHEESE SPREAD CREAM/ COTTAGE CHEESE								1 Tbs/ 1X					
24	BREAD/ROLLS: white								1 roll or 1 med slice					
25	RICE: white								½ cup or 1X					
26	PIZZA: commercial/ homemade								4 slices					
27	Breakfast cereal: refined Cornflakes, rice crispies, coco pops, strawberry pops								½ cup					
28	BREAKFAST CEREAL: high fibre e.g. All bran, highbulk, muesli, weet-bix, Pro-nutro								½ cup or 1 weet-bix or 1X					
29	Oats, taystee wheat								½ cup					
30	BREAD/ROLLS: brown								1 roll or 1 med slice					
31	BREAD/ROLLS: whole wheat/health								1 roll or 1 med slice					
32	PROVITA/ RYVITA								2 biscuits					
33	RICE: brown								½ cup or 1X					
34	MEALIE RICE/SAMP								½ cup or 1X					
35	POPCORN								1 cup or 2X					
36	LEGUMES (tinned or dry beans) e.g. baked beans, lentils								½ cup or 1X					
37	POTATO: boiled, mashed, baked								½ cup or 1X or 1 med					
38	POTATO: roasted/fried in any fat/oil (includes french-fries/slap chips)								½ cup or 1X or 1 med					
39	MARGERINE/OIL/FAT IN PREPARATION: (added to pasta, bread, potato, rice)								1 tsp					

40	SPREAD: margarine/oil/ butter on bread/ rolls/ provita/ ryvita.								1tsp					

		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
41	PEANUT BUTTER:								1 Tbs					
42	PEANUTS:								30g or 1X					
43	OTHER NUTS:								30g or 1X					
44	APPLES/PEARS								1 med or 1X					
45	CITRUS FRUIT: e.g. Oranges, grapefruit, naartjies, minolas etc.*								1 med or 1X					
46	BANANAS:								1 med or 1X					
47	PEACH/APRICOT:*								1 med or 1X					
48	WATERMELON/ SWEET MELON:*								1 large slice					
49	GUAVAS:*								1 med or 1X					
50	MANGO/ PAW-PAW/ PINEAPPLE:*								1 large slice					
51	GRAPES:*								8-10 grapes					
52	STRAWBERRIES:*								½ cup or 1X					
53	AVOCADO:*								½ med					
	*Tick No if not in season													
54	CABBAGE: (raw/ cooked)								½ cup or 1X					
55	BROCCOLI, CAULIFLOWER CARROTS (raw/cooked)								½ cup or 1X					
56	SPINACH/MAROG/ IMIFINO: (raw/ cooked)								½ cup or 1X					
57	BEETROOT								½ cup or 1X					
58	TOMATO: (raw/cooked)								½ cup or 1X					
59	ONIONS: (raw/cooked)								½ cup or 1X					
60	TOMATO AND ONION: stewed								½ cup or 1X					
61	GREEN PEAS: (raw/ cooked)								½ cup or 1X					
62	MIXED VEGETABLES: carrots, peas, mealies, beans								½ cup or 1X					
63	PUMPKIN, HUBBARD, BUTTERNUT								½ cup or 1X					

64	CORN: Mielies								1 whole or ½ cup					
65	MIXED SALAD: lettuce, cucumber, tomato, peppers, onions in any combinaton								½ cup or 1X◎					
		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
66	SWEET POTATOES								1 med/ 1/2 cup or 1X◎					
67	SUGAR (tea, coffee, porridge and cooking)								1 tsp (heaped)					
68	MUFFIN/SCONE (all other) chocolate/ poppy-seed etc								1 med or 1X ●					
69	RUSKS/BISCUITS: any								1 med or 2X ▭					
70	VETKOEK, DONUTS, SAMOOSAS, KOEKSISTER								1 med or 1X					
71	CAKE: commercial, homemade cakes/ tarts								1 med piece or 2 cookies or 1X◎					
72	JAM/SYRUP/HONEY								1 tsp					
73	SWEETS: boiled sweets, jellies, toffee								4 sweets					
74	CHOCOLATE								1 small bar/2-3 blocks					
75	TEA								1 cup					
76	COFFEE: instant/ground								1 cup					
77	ORANGE/GUAVA JUICE								1 std glass					
78	OTHER FRUIT JUICE								1 std glass					
79	MIXED JUICES : e.g. Oros, powdered mixes etc								1 std glass					
80	ENERGY DRINKS: Energade, Powerade etc								500ml					
81	Wine								125ml					
82	REGULAR BEER/ CIDERS/COOLERS								340ml (1X can/ bottle)					
83	NON-DIET FIZZY SOFT DRINKS: coke fanta/sprite								1 std glass/can					
84	DIET FIZZY DRINKS: e.g. Coke zero, coke light, sprite zero etc.								1 std glass/can					
85	SALTY CRISPS: Simba chips, Lays etc.								1 small packet					
86	SALTY BISCUITS: Salty Cracks etc.								2 biscuits					
89	Salt in cooking													

ADDENDUM C: PROTEIN EXCHANGE LIST

Food Exchange List

The 'Daily Allowance' in the list below, indicates the number of exchanges allowed from each food group per day. This has been calculated to suit your requirements. Each item in the 'Exchange Size' column is equal to 1 exchange. Choose your allocated number of exchanges from each food group per day.

Daily Allowance	Exchange Size
_____ Milk Exchanges	½ cup skim milk/low fat milk 100ml fat free sweetened yoghurt ½ cup low fat buttermilk
_____Protein Exchanges High phosphate	1 large egg/2 egg whites 2 low fat cheese wedges/1 heaped Dsp cheese spread 30 g cheese ½ cup canned pilchards
_____Protein Exchanges	30g lean meat, fish , cheese 2 heaped Dsp low fat cottage cheese ½ cup cooked lentils/beans/split peas 30 g peanuts, cashews, almonds 45g cooked, plain soya mince
_____Starch Exchanges	1 slice brown/whole-wheat bread ½ brown/whole-wheat roll ½ cup cooked porridge ½ cup high fibre cereal ½ cup Pronutro 1 Weetbix 4 Provita biscuit ½ cup cooked rice/pasta ⅓ cup cooked samp 2 cups popcorn 1 cup vegetable soup <u>Starchy Vegetables</u> 1 cup mixed vegetables with corn, peas, carrots ½ cup sweetcorn/peas
	1 small potato (100g) ½ cup sweet potato /butternut/ pumpkin (70g) Higher in potassium, do not eat more than 1 a day of these
_____Vegetable Exchanges	1 cup raw vegetables ½ cup cooked vegetables

	Mushrooms, tamato and tamato paste and sauce Higher in potassium, limit these
_____Fruit Exchanges	1 medium (tennis ball size) or, 2 small (golf ball size) fresh fruit 1 cup cubed melon 3 pineapple slices 1 cup strawberries 1 slice watermelon
	Higher in potassium, do not have more than one in a day Grapes, oranges, banana, peaches

- Don’t eat more than ¼ avocado at a time

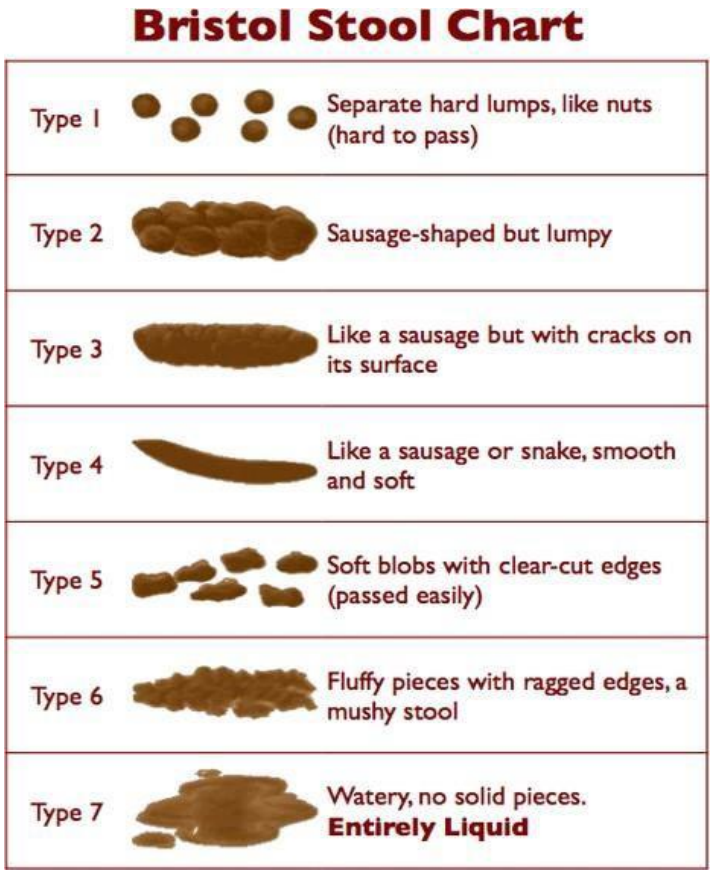
ADDENDUM D: DIETARY ADHERENCE SCORE SHEET

Participant number	Date :			
	Criteria for 1 point	Visit 4 weeks	8 weeks	14 weeks
1. Do you follow your protein allowance daily portions or with each main meal?	Yes			
2. How many high phosphate meats do you eat in a day ie eggs, liver, kidney, cheese	1			
3. How many dark coldrinks do you consume in day?	< 1			
4. How many take-away foods do you consume in a week? Burgers, chips, fried chicken	1			
5. How many burgers, polonies, sausages, viennas, crumbed boxed items, packets of soups do you eat in a week?	2			
6. How many pieces of fruit do you eat in a day	2-4			
7. How many vegetables do you eat in a day	2-4			
8. How many wholegrain foods do you eat in a day? Eg wholewheat or brown bread or crackers, oats, all bran, any other high fibre cereal, wholewheat pasta	> 2			
9. Do you add salt to your cooking (more than ¼ tsp per serving) or add at the table?	No			
10. How many other salty foods do you eat in a day ie salted chips, popcorn, packets of soups, sauces, biltong, ready made gravies	> 1			
11. how many servings of alcohol do you have in a day	< 1			
12. Do you steam, grill, boil, braise your foods daily	Yes			

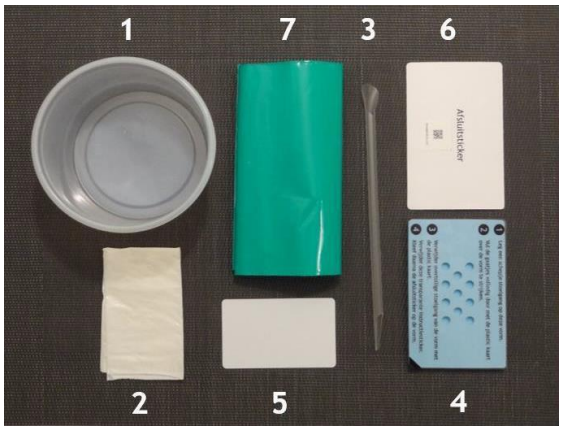
ADDENDUM E: BRISTOL SCORE SHEET

Sampling data form

Patient code :
Please write down the date and time of your sample and of your last stool.
Date of sampling :_____(DD/MM/YY)
Visit :_____(1/2/3/4)
Time of sampling:_____(24h clock)
Time of last stool :
□ less than 6 hours ago
□ between 6 and 12 hours ago
□ between 18 and 24 hours ago
□ between 24 and 36 hours ago
□ between 36 and 48 hours ago
□ more than 48 hours ago
Give your stool a score between 1 and 7 based on the description below and circle



Manual for stool sampling



1. Container *(not provided)*
2. Biodegradable plastic sheet
3. Plastic spatula
4. Device with removable sticker
5. Plastic card
6. Sealing sticker with barcode
7. Green ziplock bag

The picture above shows the material needed for stool sampling. **All materials are present in your stool sampling packet, with the exception of the container.** As a container, you can use e.g. an old ice-cream box or an old butter container (a protective plastic sheet (2) is provided to limit the exposure of the container with stool). **In addition, the sampling packet also contains a sampling form to write down some data on your sample (see p.3 of the manual), and a transparent ziplock bag to store the sampling form.**

Collect your stool sample within one week before your study visit. Read this manual carefully before you start sampling. The manual comprises 4 pages. If you prefer, you can use the provided gloves during the sampling procedure.

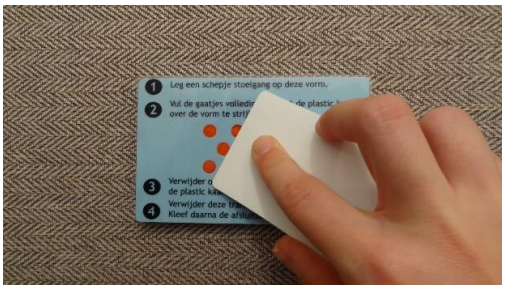
Take the container (1) and the plastic sheet (2).

Put the plastic sheet in the container and retrieve your stool (here depicted with orange play-do). The sheet keeps the container clean. **Make sure that your stool does not come in contact with urine or water.**

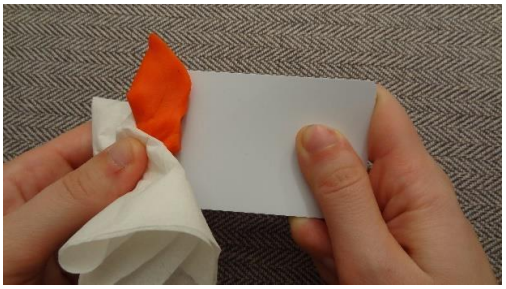
Take the plastic spatula (3), the device with the removable sticker (4), and the

Use the plastic spatula to **put a scoop of stool on the device**.

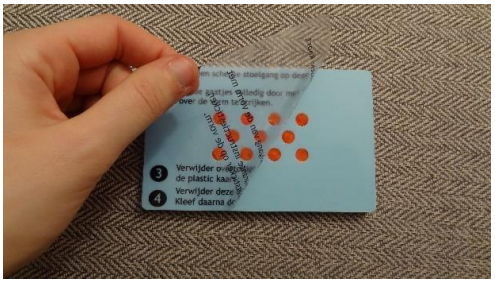
Spread your stool in the holes of the device, using the plastic card. Make sure that the holes are completely filled.



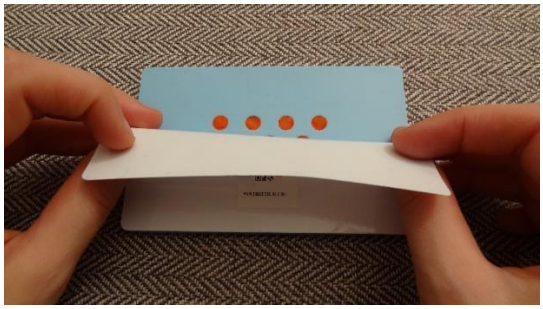
Remove excess stool from the device with the plastic card such that stool is present only in the holes.



Wipe the card with toilet paper. The toilet paper can be thrown into the toilet.



Remove the transparent sticker from the device. The surface is now clean. You can dispose the transparent sticker, the plastic card and spatula with the household waste.



Place the sticker on the clean device.

Make sure that the device is completely covered and the holes with stool are sealed.

Insert both the device and the transparent bag with the sampling form in the green bag.

Put the green bag immediately in your freezer and store it in a horizontal manner. **Your sample needs to be frozen until you hand in the sample at the collection point.** You can use an insulating pouch/a cooler bag with cooling elements or a box with ice cubes to **ensure frozentransport of your sample to the collection point.**

After the sampling procedure, you can throw away the plastic sheet together with the remaining stool, into the toilet, since the sheet is water-soluble.

ADDENDUM G: CHECKLISTS FOR FIRST AND FOLLOW-UP APPOINTMENTS

First appointment checklist
Tick the following box to ensure all avenues were covered:

Tick if done	Activities to be completed
	Add name to patient list with code
	Check patient meets inclusion criteria, check the GFR on GFR calculator
	Complete Consent forms in Duplicate
	Questionnaire
	Quantified food frequency questionnaire
	Explain infographic
	Explain portion list
	Explain stool sample collection and drop off
	Bloods taken by the nurse Doctors should do urea, creatinine, potassium, sodium For intitial visit add on CRP, cholesterol and extra bloods for centrifugation
	Patient signs for the cash
	Give the following to patient:
	Infographic
	Portion list
	Stool kit and stool chart tick list
	Cooler bag and ice packs
	R50 cash for transport Explain the voucher will be given when stool sample is brought back

Follow-up appointment checklist
Tick the following box to ensure all avenues were covered:

Activities to be completed	Tick if done	Tick if done	Tick if done
Double check for missing information			
Do anthropometry			
Clinical			
Quantified food frequency questionnaire			
Diet adherence			
Diet patterns			
Symptoms questions			
Bloods taken by the nurse Urea, creatinine, potassium, sodium CRP, cholesterol and extra bloods for centrifugation			
Patient signs for the cash			
Give the following to patient:			
R50 cash for transport Voucher			
Re-inforce diet			
Issue product if in intervention group			
Date for following appointment for collection			
Voucher will be slightly less			

Thank you!

“ It always seems impossible until its done ”

Nelson Mandela